

**The Effects of Acute Aerobic Exercise on Inflammatory
Markers and Mood in Individuals with Multiple Sclerosis and
Spinal Cord Injury**

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Abstract

The purpose of this study was to determine if acute exercise-induced reductions in pro-inflammatory cytokines contribute to exercise-induced improvements in mood in individuals with spinal cord injury (SCI) and multiple sclerosis (MS). Thirteen participants completed a single 30-minute bout of moderate-intensity exercise at 60% of their pre-determined VO_2 peak. Mood was assessed before, immediately after, and one-hour post-exercise, via the Profile of Mood States Questionnaire (POMS). Blood samples were taken before, immediately after, and one-hour post exercise, and subsequently analyzed for serum pro-inflammatory cytokines (IL-6, IFN- γ , and TNF- α), as well as the amino acids tryptophan (TRP) and kynurenine (KYN). Previous work from our lab has shown that chronic reductions in pro-inflammatory cytokines via diet improve mood via the kynurenine pathway, which is integral in serotonin production. Following the bout of exercise, there was a significant improvement in mood as shown by a reduction in the total POMS questionnaire score from pre to post-exercise (32.5 ± 25.8 to 22.4 ± 21.9 ; $p=0.025$), as well as pre to one hour post-exercise (32.5 ± 25.8 to 21.6 ± 25.0 ; $p=0.008$). Subscale analysis showed significant reductions in the Tension, Depression and Anger components of the POMS from pre to post-exercise and from pre to one-hour post-exercise. Regarding pro-inflammatory cytokines, there were no exercise-induced changes in IL-6, but there was a significant main effect for time for TNF- α (with post-hoc analyses showing significant reductions from pre to one-hour post-exercise) and a trend for a main effect for time for IFN- γ ($p=0.06$). There were no changes in TRP, KYN or KYN/TRP. There were no correlations between changes in mood and changes in cytokines when all participants were analyzed. However, when considering only the

participants that responded to exercise with an improved mood ($n=7$), there were significant correlations between exercise-induced changes in depression and exercise-induced changes in IL-6 ($r=0.853$; $p=0.031$) and a trend for a correlation with exercise-induced changes in TNF- α ($r=0.722$; $p=0.067$). These results suggest that exercise-induced changes in mood may be partially accounted for by exercise-induced changes in pro-inflammatory cytokines in individuals with SCI and MS, but not through a kynurenine pathway-dependent manner.

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Table of Contents

Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	v
List of Abbreviations.....	viii
List of Tables.....	ix
List of Figures.....	x
List of Appendices.....	xi
I. Introduction.....	1
II. Literature Review.....	4
2.1.0 Background and Epidemiology of Multiple Sclerosis.....	4
2.1.1 <i>Epidemiology and Demographics</i>	4
2.1.2 <i>Etiology</i>	5
2.1.3 <i>Types of MS</i>	5
2.1.4 <i>Pathology</i>	6
2.2.0 Background and Epidemiology of Spinal Cord Injury.....	7
2.2.1 <i>Epidemiology and Demographics</i>	7
2.2.2 <i>Etiology</i>	7
2.2.3 <i>Anatomy of the Spinal Cord</i>	8
2.2.4 <i>Injury Level</i>	8
2.2.5 <i>Severity</i>	9
2.3.0 Immunoregulatory Function.....	9
2.3.1 <i>Cytokine Production</i>	9
2.3.2 <i>The Immune System</i>	10
2.3.3 <i>Innate Immunity</i>	10
2.3.4 <i>Adaptive Immunity</i>	11
2.3.5 <i>The Inflammatory Response</i>	12
2.3.6. <i>Cytokine Homeostasis</i>	13
2.4.0 Immune Dysfunction & Chronic Inflammation Following MS & SCI.....	14

2.4.1 <i>Autonomic Nerve Damage</i>	15
2.4.2 <i>Endocrine Dysfunction</i>	16
2.5.0 <i>Depression</i>	17
2.5.1 <i>Background</i>	17
2.5.2 <i>Mechanisms Underlying Depression</i>	18
2.5.3 <i>Treatments</i>	23
2.5.4 <i>Exercise as a Treatment Option</i>	24
2.6.0 <i>The Effect of Acute Exercise on Cytokines</i>	26
2.7.0 <i>Conclusion</i>	30
III. <i>Purpose and Hypothesis</i>	31
3.1 <i>Statement of Purpose</i>	31
3.2 <i>Hypothesis</i>	31
IV. <i>Overview of Study Design</i>	32
4.1 <i>Study Design and Participants</i>	32
4.2 <i>Exercise Protocol</i>	33
4.3 <i>Quantification of serum inflammatory markers and amino acids</i>	35
4.4 <i>Assessment of Mood</i>	36
4.5 <i>Assessment of Blood Markers</i>	36
4.6 <i>Statistical Analysis</i>	37
V. <i>Results</i>	39
5.1 <i>Exercise Adherence</i>	39
5.2 <i>Changes in POMS</i>	39
5.3 <i>Changes in Inflammatory Mediators</i>	40
5.4 <i>Changes in Amino Acids</i>	41
5.5 <i>Correlations between POMS and Blood Markers</i>	42
5.6.0 <i>Subgroup Analysis</i>	44
5.6.1 <i>Subgroup Changes in POMS</i>	45
5.6.2 <i>Subgroup Changes in Inflammatory Mediators</i>	46
5.6.3 <i>Subgroup Changes in Amino Acids</i>	47
5.6.4 <i>Subgroup Correlations between POMS and Blood Markers</i>	48

VI. Discussion.....	51
6.1 Major Findings.....	51
6.2 Clinical/ Physiological Significance.....	53
6.3 Limitations.....	55
6.4 Future Directions.....	56
6.5 Conclusions.....	59
Appendices.....	81

List of Abbreviations

ACTH – Adrenocorticotropin
AIS – ASIA (American Spinal Injury Association) Impairment Scale
ANS – Autonomic Nervous System
APC – Antigen Presenting Cells
BBB – Blood Brain Barrier
CNS – Central Nervous System
CRH – Corticotrophin-releasing hormone
EDSS – Expanded Disability Status Score
FES – Functional Electrical Stimulation
HPA Axis – Hypothalamic-Pituitary-Adrenal Axis
IDO – Indoleamine 2,3-dioxygenase
IFN – Interferon
IL – Interleukin
KAT – Kynurenine aminotransferase
KMO – Kynurenine 3-monooxygenase
KYN – Kynurenine Pathway
KYN-KYNA – Kynurenine-kynurenic acid
KYN-NAD – Kynurenine-nicotinamide adenine dinucleotide
KYNA – Kynurenic acid
MDD – Major depressive disorder
MHC – Major Histocompatibility Complex
MS – Multiple Sclerosis
NDMA – N-methyl-D-aspartate
NEFA – Non-esterified fatty acid
NK cell – Natural Killer cell
PAMP – Pathogen-associated molecular patterns
PNS – Parasympathetic Nervous System
POMS – Profile of Mood States
PPMS – Primary Progressive Multiple Sclerosis
PRMS – Primary Relapsing Multiple Sclerosis
PwMS – People with Multiple Sclerosis
QUIN – Quinolinic acid
RRMS – Relapse Remitting Multiple Sclerosis
SCI – Spinal cord injury
SERT – Serotonin transporters
SNS – Sympathetic Nervous System
SPMS – Secondary progressive multiple sclerosis
SSRI – Selective serotonin reuptake inhibitors
TDO – Tryptophan 2,3-dioxygenase
Th – T Helper cell
TNF – Tumour Necrosis Factor
TRP – Tryptophan
3-HK – 3-hydroxykynurenine

List of Tables

IV. Methodology

Table 1: Participant Characteristics.....	33
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V. Results

Table 2: Change in Inflammatory Mediators.....	41
Table 3: Change in Amino Acids.....	42
Table 4: Inflammatory Mediators and Mood Pre to Post Correlations.....	43
Table 5: Inflammatory Mediators and Mood Pre to 1 Hour Post Correlations.....	44
Table 6: Subgroup Change in Inflammatory Mediators.....	47
Table 7: Subgroup Change in Amino Acids.....	48
Table 8: Subgroup Inflammatory Mediators and Mood Pre to Post Correlations.....	49
Table 9: Subgroup Inflammatory Mediators and Mood Pre to 1 Hour Post Correlations.....	50
Table 10: Pilot Participant Characteristics.....	91
Table 11: Pilot Feeling Scale Questionnaire.....	92
Table 12: Pilot Brief Pain Inventory.....	92
Table 13: Pilot Neuropathic Pain Questionnaire.....	92

List of Figures

II. Literature Review

Figure 1: Peripheral and central kynurenine pathway interactions.21

Figure 2: Inflammatory mechanisms of depression.....22

Figure 3: The proposed role of acute exercise on the kynurenine pathway29

IV. Methodology

Figure 4: Protocol Flowchart.....38

V. Results

Figure 5: Change in POMS Scores across Time points.....40

Figure 6: Subgroup Changes in POMS Scores across Time points.....46

Figure 7: Pilot Profile of Mood States.....91

List of Appendices

1. Raw Data.....	80
a. POMS Scores.....	80
b. Blood Markers.....	82
2. Kurtzke Expanded Disability Status Scale (EDSS).....	85
3. AIS Scale.....	86
4. Profile of Mood States Questionnaire.....	87
5. Ethical Approval.....	89
6. Pilot Data.....	90

I. Introduction

The diagnosis of multiple sclerosis (MS) or a spinal cord injury (SCI) is a traumatic life altering event, requiring significant modifications in many aspects of a person's life. Along with the necessary lifestyle changes, there are many alterations that happen within multiple systems of the body[1, 2]. As the body requires a series of bi-directional communications within systems to sustain homeostasis, damage to one system can have a lasting negative effect on another. By extension, various systems can thus contribute to the development of a disorder.

Neural dysfunction that accompanies MS and SCI can contribute to impairments within both the immune and endocrine systems. Due to the bi-directional nature of these communication pathways, dysfunction within the immune system can in turn have a large impact for further deficiencies within the neural and endocrine systems via multiple inflammatory mechanisms[3]. Pro-inflammatory cytokines can directly influence the nervous system by acting on vagal afferents, or by crossing the blood brain barrier (BBB) via leaky sites or specialized transporters[4]. Hormonal secretion of the hypothalamic-pituitary-adrenal (HPA) axis can also be influenced by pro-inflammatory cytokines due to overstimulation of its receptors[5]. Additionally, pro-inflammatory cytokines may indirectly cause dysfunction within these systems by altering the regulation of enzymes; resulting in shifts in metabolic pathways and an imbalance of key neuroactive compounds[6]. Systemically elevated levels of pro-inflammatory cytokines are commonly referred to as chronic inflammation which can contribute to further neural, metabolic, and behavioural disorders[7]. This creates a vicious cycle, as these disorders have been shown to further contribute to elevated levels of pro-inflammatory cytokines.

Both MS and SCI populations have been shown to have elevated basal levels of pro-inflammatory cytokines such as IL-1 β , IL-6, IFN- γ and TNF- α in comparison to able-bodied populations, potentially contributing to the increased rates of metabolic and behavioural disorders within these populations[8, 9].

There has been consistent evidence that depressed populations demonstrate elevated levels of pro-inflammatory cytokines such as IL-1 β , and TNF- α [10]. As MS and SCI populations are already exposed to elevated levels of pro-inflammatory cytokines due to dysfunction in other systems, they are more susceptible to depression as illustrated with depression rates 2-10 times higher than that of the general population[11–13]. Current treatment strategies for depression have been shown to be ineffective in negating depressive symptoms in a percentage of people, as the drug therapies typically utilized only address downstream targets[14]. In addition to the negative side effects that can be associated with these drug therapies, alternative approaches should be explored for addressing depression and depressed mood.

Exercise has long been shown to be effective in reducing the secondary health risks associated MS and SCI[15, 16]. A single bout of moderate aerobic exercise has been shown to be uniquely anti-inflammatory in that it seems to bypass the traditional increase in pro-inflammatory cytokines seen in immune responses, and instead increase the production of anti-inflammatory cytokines such as IL-6, IL-10, and IL-1RA[17, 18] As anti-inflammatory cytokines have been shown to inhibit the production of pro-inflammatory cytokines and indirectly affect numerous enzymes and receptors, exercise offers a distinctive role as a holistic means to reduce inflammation. Additionally, moderate intensity exercise has been consistently demonstrated to improve the mood of

healthy, depressed, and special populations[19–21]. Due to the established link between inflammation and mood in long-term exercise studies, questions arise for the prospective role that acute exercise-induced alterations in the anti-inflammatory environment contribute to mood modifications. There remains a large gap in the literature regarding the physiological mechanism behind the demonstrated exercise-induced mood changes in any population group. Therefore, the exploration of the potential acute relationship between exercise-induced changes in inflammation and mood is the focus of this thesis.

II. Literature Review

2.1.0 Background and Epidemiology of Multiple Sclerosis

2.1.1 Epidemiology and Demographics

The incidence rate of multiple sclerosis (MS) in Canada is 240 per 100000, making it the highest incidence rate in the world [22]. These rates are increasing, especially in women [23]. Of these individuals, approximately 85% are first diagnosed with relapse-remitting multiple sclerosis (RRMS), meaning that they have temporary relapses or exacerbations of deficit with full or close to full recovery in between relapse periods [24]. Due to the varying size and locations of an individual's lesions, many people are affected in different ways by the disease. In regards to the degree of disability, the progressive nature of MS results in variable symptom severity (as measured by the Expanded Disability Status Scale (EDSS)) throughout disease progression. Tremlett et al., (2006) reported that on average, MS progresses from the onset of diagnosis to an EDSS score of 6 over the course of 27.9 years; where a score of 6 indicates the use of a walking aid to walk 100m.

The median age of onset of the disease is 28 years, with most onsets occurring between the ages of 18-40 [25]. It is more commonly diagnosed in women, at a ratio of 3:1. Despite MS being more common in women, men's disease progression and disability onset tends to be more severe [26]. The mortality rates of those aged 30-39 have been decreasing while mortality rates in the age groups 60-69, 70-79, and 80+ have increased since 1975, most likely as a result of advancements in health care and health promotion that keep people alive after the initial diagnosis of the disease [27].

2.1.2 Etiology

MS is of an unknown etiology. The leading theory into the cause of the disease is a combination of environmental and genetic factors. Earlier research has indicated that there is a positive correlation between higher MS incidence rates and the distance one lives from the equator. Work done by Hernàn, Olek, and Ascherio (1999) has disputed this correlation by attributing it to limited resources and information from the countries closer to the equator. While geographic latitude is no longer thought to influence the incidence of MS, regional variance has been shown in larger countries such as Canada [23, 28, 29].

2.1.3 Types of MS

There are four distinct forms of MS, with 85% of people originally diagnosed with RRMS, making it the most common form of MS. RRMS is characterized by episodes of acute neurological deterioration called relapses, which are then followed by a period called remission, where there is close to a full recovery to prior relapse state and function.

Over the duration of their disease, two-thirds of individuals with RRMS will progress into secondary progressive multiple sclerosis (SPMS) [25]. SPMS is characterized by steady deterioration with or without the presence of relapses or remissions.

The third form of MS is primary progressive multiple sclerosis (PPMS). It rarely occurs, diagnosed only in 10% of all MS cases. This form of MS does not have relapses and remissions, but starts with a slow, progressive deterioration from the onset of

diagnosis. Contrary to most forms of MS, where there are higher rates in women, PPMS affects the sexes roughly equally[24], and the age of onset is 10 years later than the more common forms of MS [30]. While the age of onset for PPMS may be later in life, the disease progression is much faster, with this group reaching an EDSS score of 6 at a younger age[26].

The final, and most rare type of MS is primary-relapsing multiple sclerosis (PRMS), occurring in only 5% of the MS population. The disease progression is steady from the beginning of the diagnosis, with clear relapses but no remission periods.

2.1.4 Pathophysiology

The pathophysiology of MS is typically associated with demyelinated lesions with partial axonal preservation. Lesions can be found in the white matter of the ventricles, the brainstem, spinal cord, and optic nerve. MS lesion size and location vary in every patient, making no two cases the same. The type of MS the person has is also important in the pathology of the disease. Acute and relapsing types of MS have a larger inflammatory profile consisting of major histocompatibility complex (MHC) Class 1-restricted CD8⁺ T cell expression within the lesions. The activated T cells and macrophages travel through the compromised blood brain barrier (BBB), which then target the myelin sheaths of the nerve axons [31].

Remyelination of these damaged sites has been seen in earlier stages of MS during remission periods. However, the rate of remyelination will eventually slow or stop all together during the progressive components of the disease. The exact cause of this attenuation is thought to be due to fewer oligodendrocyte progenitor cells being signaled

to the demyelinated site [32]. In SPSS and PPSS the inflammatory profile is much more limited. Instead of an increase in new lesions and further demyelination of the white matter, lesions will instead slowly expand in size, damaging the surrounding areas of the nerve and thus causing further functional decline [31].

2.2.0 Background and Epidemiology of Spinal Cord Injury

2.2.1 Epidemiology and Demographics

The incidence rate of spinal cord injuries are steadily increasing, with rates of 35 per million population in Canada [33]. The age of onset for a spinal cord injury has traditionally been the highest between the ages of 16-29. Recently, a second spike has risen for the 65+ age bracket, with many of these injuries coming due to a fall. As a result of this second spike, the mean age of people with a spinal cord injury has shifted from 32 to 42 years of age between 1986-2006 [34]. Even with a shift in the average age of people living with SCI, young males outnumber female incidence rates at a ratio of 3:1 [34].

2.2.2 Etiology

In Canada, motor vehicle accidents (MVA) have historically been the most common cause of SCI, with numbers ranging between 50-60% of all cases. Falls now constitute the second highest cause of SCI, and these rates are constantly increasing to the point where some studies rate them as more causative than MVA [33]. This is evident in populations over 65, as falls represent 63% of the cause of SCI in this age bracket [35]. Other causes of SCI such as sport injuries and injuries due to violence have been in decline, and only account for a combined 20% of all SCI [36].

2.2.3 Anatomy of the Spinal Cord

The spinal cord is contained within the protective vertebral column and cushioned by the cerebral spinal fluid surrounding it. It is essential to relaying motor and sensory information between the brain and all parts of the body. The spinal cord is composed of ascending tracts that relay sensory information such as proprioception, touch, pain, and temperature, and descending tracts that relay motor information to efferent nerves within the body. The inner grey matter is composed of interneurons and neuronal cell bodies. The amount of white and grey matter varies depending on the spinal segment, with larger amounts of grey matter appearing in the cervical and lumbosacral segments [37].

2.2.4 Injury Level

In general, the level of SCI can be classified into two categories, tetraplegia and paraplegia. Tetraplegia results from an injury to the cervical region of the spinal cord and is characterized by the loss of sensory and/or motor contributions to the upper extremities, trunk, lower extremities, and pelvic organs. If the lesion occurs in the thoracic, lumbar, or sacral segments of the spinal cord, paraplegia results, which is characterized by normal upper extremity innervation. However, the trunk, lower extremities, and pelvic organs may be affected depending on the location of the lesion [37]. Regarding the level of injury, 40-75% are cervical injuries, 9-11% thoracic, and 10-26% sacral-lumbar injuries [33, 38, 39].

2.2.5 Severity

There is a large range in the severity or completeness of an SCI. When there is no motor and sensory function at some point below the injured site, the injury is termed complete. Clinically, a complete injury will be determined by the absence of sensory and motor capabilities in the 4th and 5th sacral regions of the spinal cord. In the cases where there is partial motor and/or sensory function maintained, including the 4th and 5th sacral segments, the injury is termed incomplete. It is estimated that half of all injuries are incomplete, with percentages increasing due to improved health care[36, 38]. The concept of injury severity can be further specified with the 5-point (A-E) ASIA Impairment Scale (AIS; where ASIA stands for American Spinal Injury Association). AIS A denotes a complete injury, while AIS E denotes a SCI with full recovery of motor and sensory function. AIS scores of B, C, and D correspond to an incomplete injury with increasing sensory and motor functionality. (See appendix for further definitions).

2.3.0 Immunoregulatory Function

2.3.1 Cytokine Production

Cytokines are small proteins that assist with the communication between cells for the purpose of immunoregulation. They can be produced within the brain, spinal cord, and skeletal muscle by immune cells such as T-cells and macrophages. The term cytokine is very broad and covers a multitude of cells that can have roles in autocrine, paracrine, or endocrine actions [40–43]. A single cytokine may also have pleiotropic characteristics, meaning that they can exert multiple actions, and are often produced in a cascade [43].

2.3.2 The Immune System

The immune system comprises a large collection of cells and molecules that work together to defend the body from foreign pathogens. The immune system utilizes the complementary actions of two separate but interdependent components; the innate and the adaptive (or acquired) immune systems, to protect the body by identifying, attacking, and destroying the potentially harmful pathogens [44]. The innate system is the first line of defence, and it is non-specific in that it will fight pathogens in the same way, regardless of whether or not the system has encountered them before. If the innate system proves ineffective, the adaptive immune system will be activated and use pathogen-specific strategies to attack and destroy [42].

2.3.3 Innate Immunity

The innate system is immediately activated when an infectious agent attempts to enter the body. All immune defenses that lack an immunologic memory are considered to be part of the innate immune system [45]. Cells called phagocytes, which include neutrophils, macrophages, and dendritic cells, engulf foreign pathogens through a process called phagocytosis in which the pathogen is destroyed. Another aspect of the innate immune system is the production of natural killer (NK) cells. Natural killer cells are important in the disintegration of viral and cancer cells by breaking up the cell membrane of these cells via pore-forming proteins [42, 45]. The innate system utilizes recognition molecules called toll like receptors (TLR) to sense the pathogen-associated molecular patterns (PAMPs) of bacteria, indicating to the system that the pathogen is foreign. Accordingly, there is activation of both phagocytes of the innate system, (neutrophils and

macrophages) cytokine production, and antigen presenting cells (APC) of the adaptive immune system [42, 46].

2.3.4 *Adaptive Immunity*

Unlike the innate system, the adaptive immune system utilizes past experiences to produce specific antigens via lymphocytes to destroy invading pathogens. It can further be categorized into humoral or cell mediated immunity [42].

Humoral immunity defends the body from extracellular pathogens by the activation of circulating immunoglobulins. During the innate immunity response, phagocytes including monocytes, macrophages, and dendritic cells engulf the pathogen and destroy it. The activated TLRs on the surface of the antigen presenting cells stimulate increased expression of major histocompatibility complex (MHC) II proteins [47]. Left over parts of the destroyed pathogen are then accepted and they can be presented to naïve or memory T-cells that have never been presented with the antigen before, initializing a primary immune response. This causes proliferation of more T-cells that will now be able to recognize that specific antigen for future encounters [48]. Memory T-cells (T-cells that have previously encountered that specific antigen) can produce a faster, and more efficient secondary response. T-helper (Th) cells can coordinate this secondary response via the release of cytokines to activate other immune cells. This stimulates mature B cells to proliferate and differentiate into immunoglobulin-secreting plasma cells [48]. Both B and T cells circulate throughout the body to provide antigen-specific surveillance of any invading pathogens. While immunoglobins (or antibodies) are unable to destroy the antigen by themselves, they either block the toxic release of some antigens through a

process known as neutralization, or they provide a road-block by clumping together with the antigen to slow it down and will call on phagocytes to destroy the antigen in a process known as agglutination [42].

If the pathogen has moved intracellularly, humoral immunity is no longer effective and the body must now utilize cell-mediated immunity. Cell mediated immunity is mediated by activated effector T cells and macrophages for intracellular pathogens and infected host cells. When T-helper cells bind to the MHC complex proteins of macrophages, a specific antigenic determinant is displayed. This stimulates the macrophage to release the cytokine IL-1, which then stimulates T cells to grow and divide. The activated T cells then release IL-2, which further aids in the growth and dividing of more T-helper and Tc (T-cytotoxic) cells. The T-cytotoxic cells can recognize the same antigenic determinant displayed on the original MHC complex protein. T-cytotoxic cells will then release perforin, causing death of the infected host cell by lysis. The left over cell debris is then ingested and digested by phagocytes [42].

2.3.5 The Inflammatory Response

Cytokines play an important role in the body's defense against invading pathogens. For both the innate and adaptive immune systems, cytokines assist with healing and/or preventing tissue damage by sending signals to other immune cells to proliferate and travel to the damaged area [40]. While the immune system is generally effective, an inflammatory response may occur to prevent the spreading of the pathogen and/or heal the affected area. Once a foreign pathogen is detected there is a release of histamine by mast cells in the affected area. This causes both vasodilation in surrounding

arterioles, which brings more blood to the affected area, and an increase in capillary permeability [42]. The increase in blood flow is accompanied by an influx of phagocytic leukocytes and plasma proteins. Externally, the increase in blood flow leads to redness and emitted heat from the affected area. The increased capillary permeability allows the protein fibrinogen into the area where it is converted into fibrin. Fibrin walls off the invading pathogen by forming interstitial fluid clots, which prevents the spreading of the pathogen and allowing time for phagocytes to arrive to the area [42]. Chemical mediators known as chemokines send signals to attract more phagocytes to the site, which further assist in the break down of the pathogen. In addition to more phagocytes being recruited to the affected site, new leukocytes are proliferated in the bone marrow from cytokine release from activated T-cells. The combination of increased recruitment and proliferation of phagocytes leads to the removal of the pathogen and clears the area for tissue repair [42].

Cytokines can modify brain behaviour and function by hormonal and neural avenues, and may access the brain by travelling through leaky sites in the blood brain barrier (BBB) via circumventricular organs, and by using specific transporters. Cytokines can also communicate with the brain by acting on the vagal afferent pathways [4].

2.3.6. Cytokine Homeostasis

Cytokines work to keep the body in immune homeostasis by both promoting and inhibiting other immune cells. This makes them very difficult to categorize as some have multiple roles, while others can be redundant in their actions [40, 41]. A common way to

categorize cytokines is by placing them into two categories that indicate their role in the inflammatory process. Pro-inflammatory cytokines promote immune responses such as proliferation and recruitment of immune cells in the instance of an invading foreign pathogen [40]. In contrast, once the pathogen is phagocytized and the healing process begins, anti-inflammatory cytokines are released to inhibit further production of pro-inflammatory cytokines, and by extension, help bring cytokines back to prior levels [40]. This balance in cytokine levels is important for a maintained immune homeostasis, which is seen in healthy individuals. Many special populations, including those with MS and SCI may have a dysfunction in this homeostasis, which can lead to negative health effects.

2.4.0 Immune Dysfunction & Chronic Inflammation Following MS & SCI

The complex bi-directional relationships between the nervous, endocrine and immune systems contribute to chronic inflammation, as dysfunction within one system can alter homeostasis within another. The loss of autonomic innervation of lymphoid organs and corresponding dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis contribute to the higher rates of chronic inflammation in both people with MS and SCI [3]. It is unknown as to what system initially influences the others, but dysfunction within one system influences dysfunction within others, and vice-versa, thus creating a vicious cycle. Ultimately, the resulting inflammation accumulating in the body may lead to other secondary health complications, such as urinary tract infections, obesity, cardiovascular disease, and psychological disorders such as depression [49, 50].

2.4.1 Autonomic Nerve Damage

The autonomic nervous system, consisting of the sympathetic and parasympathetic nervous systems (SNS and PNS) acts as a neuromodulator of the immune system in the attempt to maintain physiological homeostasis [51]. In the SNS, preganglionic fibres originating from the spinal cord between T1-L2 synapse in peripheral autonomic ganglia. Postganglionic fibres reach their effector lymphoid organs such as the thymus, spleen, adrenal medulla, and lymph nodes, where they release catecholamines, such as the neurotransmitter norepinephrine (NE) for direct communication with lymphocytes [52]. Catecholamines have been shown to increase the production of anti-inflammatory cytokines such as IL-4, which act to suppress the continued production of pro-inflammatory cytokines. Damage to the SNS may also contribute to reduced immune function due to a loss of afferent signaling from the adrenal gland to the hypothalamus. Impairments to this afferent pathway may reduce activation of the hypothalamic nuclei, which may ultimately induce dysfunction within the HPA axis of the endocrine system [1].

The PNS works antagonistically to the SNS and has preganglionic fibres originating from the vagus nerve and the S2-S4 region of the spinal cord which synapse with postganglionic fibres that release acetylcholine (ACh) [51]. The PNS may have a role in peripheral inflammatory responses via the cholinergic anti-inflammatory pathway, where vagal afferent and efferent pathways contribute to inhibiting TNF- α levels [53].

Damage to the ANS restricts the system's ability to monitor and inhibit the release of pro-inflammatory cytokines such as TNF- α and IL-1 β , and promote the

production of anti-inflammatory cytokines, as shown in both SCI and MS populations [54–57]. This further promotes the chronically elevated levels of circulating pro-inflammatory cytokines within the body, potentially leading to further dysfunction within other systems.

2.4.2 Endocrine Dysfunction

The Hypothalamic-Pituitary-Adrenal (HPA) axis acts as a hormonal pathway for the CNS to regulate the immune system. Dysfunction of the HPA axis occurs when there is damage to central nervous system, causing an imbalance in the regulation of immune cells [5]. Corticotrophin-releasing hormone (CRH) is secreted from the hypothalamus, which stimulates the expression of adrenocorticotropin (ACTH) from the anterior pituitary gland. ACTH activates the adrenal gland and stimulates the expression and release of glucocorticoids, such as cortisol. Glucocorticoids modulate cytokine expression by suppressing the release of pro-inflammatory cytokines and upregulating anti-inflammatory cytokines [5]. This induces a shift from a T-1 helper (Th1) to T-2 helper (Th2) pattern of immunity, mostly due to the downregulation of pro-inflammatory cytokines, which permits the dominance of Th2 anti-inflammatory cytokines to take over. When disruption of this pathway occurs, as in the instance of a traumatic spinal cord injury or multiple sclerosis, dysfunction in the HPA axis can result either in a lack of glucocorticoid production, or desensitization of the receptors [1, 3]. This shifts the immune system back from a Th2 to Th1 dominance, where the anti-inflammatory response is blunted and there is an increase in pro-inflammatory cytokines [58]. The increase in the production of pro-inflammatory cytokines and decrease in anti-inflammatory cytokines combine to produce a state of chronic inflammation in both

population groups, which can lead to other secondary health issues [8, 9, 59]. Elevated pro-inflammatory profiles have been found in both spinal cord injury and multiple sclerosis populations in comparison to control populations [8, 9]. Positive correlations have been shown such that the higher the SCI level, the greater the degree of pro-inflammatory cytokines [9]. Furthermore, positive correlations have also been shown such that the further the disease progression in MS, the greater the degree of pro-inflammatory cytokines [8]. Interestingly, higher levels of anti-inflammatory cytokines such as IL-4 and IL-10 were also found in the MS groups, possibly as a strategy to downregulate the elevated pro-inflammatory cytokines such as IL-1 β and TNF- α .

2.5.0 Depression

2.5.1 Background

Both the MS and SCI populations have an increased risk of depression [12, 60]. Depression is characterized as a mood disorder negatively affecting how one feels, thinks, and acts [61]. Symptoms must last for longer than two weeks and can range from feelings of sadness, worthlessness, anger, fatigue, suicidal tendencies, anxiety, and loss of enjoyment in normally pleasurable activities.

Approximately 25% of the MS population has been reported to suffer from depression, with some studies reporting rates as high as 50% [12]. In the SCI population, depression rates have been estimated between 7-31% [60]. These rates contrast with the 4.8% rate of depression that is typically reported in the able-bodied [62], which is a great concern for both population groups and calls for interventions in prevention and treatment for the affected people. Suicide rates are also much higher in individuals with

MS and SCI, as it has been reported that suicide rates are 2-7.5 times greater in people with MS [11] and 3-5 times greater in people with SCI in comparison to the general population [63, 64]. People with depressed mood may present with similar symptoms to clinical depression with the caveat that it does not last up to two weeks [61]. Mood, as opposed to emotions, are typically longer lasting and more generalizable, as they may be temporal with many stressors contributing to one's overall mood [65]. Acute stressors such as rejection, mourning, or anxiety may help contribute to feelings of depressed mood [66], without it fully evolving into depression.

Being diagnosed with MS or an SCI is a life-altering event that requires many lifestyle changes by the individual and his or her family. Coming to terms with one's new life can influence psychological well-being on its own. However, it is important to note the comprehensive range of underlying mechanisms behind depression and depressed mood in MS and SCI populations.

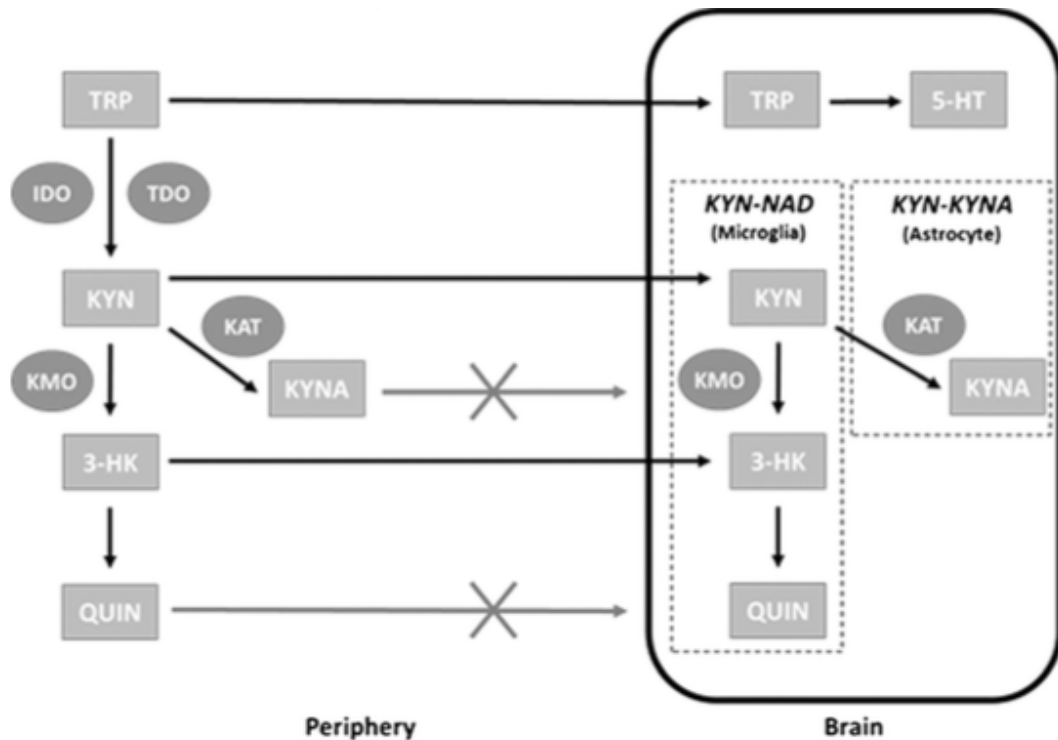
2.5.2 Mechanisms Underlying Depression

Chronically elevated inflammatory profiles may lead to depressive symptoms as a result of cytokine influences on the neuroactive compounds of the kynurenine pathway (KYN) [67, 68]. This metabolic pathway, in which tryptophan (TRP) is degraded into other metabolites (with the end result being NAD⁺) [69, 70] offers a potential mechanism such that any dysfunction within this pathway will have important implications for depression and depressed mood. Tryptophan is essential for serotonin (5-HT) production within the brain. However, alternatively, tryptophan can also be oxidized by the enzymes

2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO), to become kynurenine (KYN). KYN will then follow one of two pathways; the kynurenine-kynurenic acid (KYN-KYNA) pathway within astrocytes or the kynurenine-nicotinamide adenine dinucleotide (KYN-NAD) pathway, which occurs in microglia cells. Within the KYN-KYNA pathway, KYN is acted upon by the enzyme kynurenine aminotransferase (KAT), where it becomes the neuroprotector kynurenic acid (KYNA). Following the KYN-NAD pathway, KYN is acted upon by the enzyme kynurenine 3-monooxygenase (KMO) where it becomes 3-hydroxykynurenine (3-HK), later to become the neurotoxin quinolinic acid (QUIN) and finally NAD^+ [69, 70]. Under normal circumstances, the kynurenine pathway is well regulated and a balance is found between the neuroprotector KYNA and the neurotoxin QUIN [69]. However, when there is an increased basal amount of pro-inflammatory cytokines, there is a corresponding upregulation of IDO, which increases the rate of degradation of tryptophan to KYN, reducing the amount that will travel to the brain to synthesize serotonin [14]. With more tryptophan being degraded by IDO, there is an indirect increase in the levels of QUIN. Elevated QUIN levels cause an over-activation of N-methyl-D-aspartate (NDMA) receptors leading to hippocampal atrophy and HPA axis dysfunction [69, 70]. Atrophy within the hippocampus has shown in depressed populations, although if depression causes hippocampal atrophy or hippocampal atrophy causes depression remains inconclusive [71, 72]. While a healthy HPA axis can produce glucocorticoids to activate the negative feedback loop to effectively turn off further production from the adrenal gland, atrophy within the HPA axis may reduce this negative feedback loop, thus over-stimulating the HPA axis and causing dysfunction within the

region. Elevated levels of pro-inflammatory cytokines have been consistently found in people with depression[73], with these mechanisms being the potential causative link.

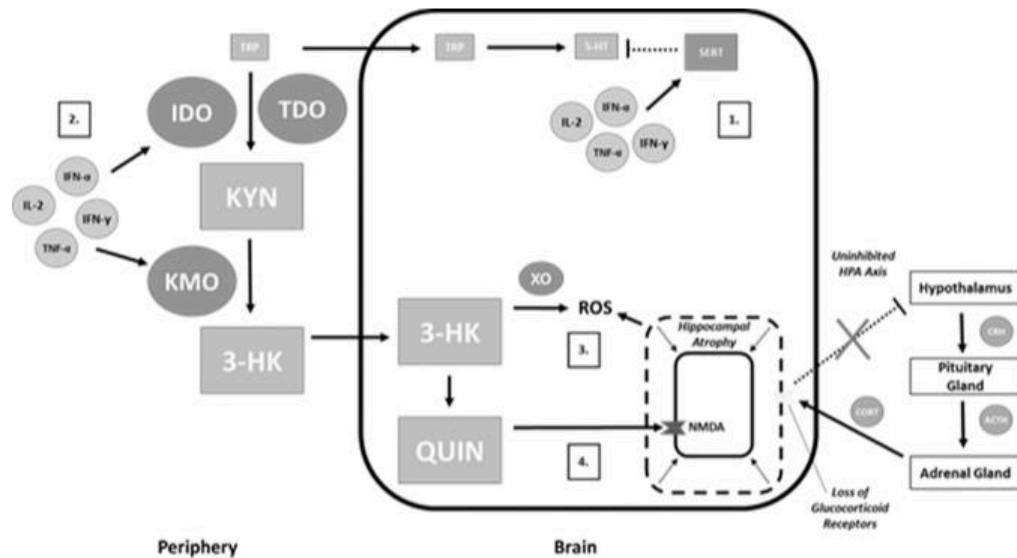
An additional mechanism limiting the availability of serotonin is through the upregulation of serotonin transporters (SERT) by pro-inflammatory cytokines. SERT mediates reuptake of serotonin and helps maintain homeostasis. However, higher levels of pro-inflammatory cytokines such as TNF- α and IL-1 β , as seen in chronically inflamed populations, have been shown to amplify the up-regulation of SERT proteins within the brain, leading to an increased reuptake of serotonin and corresponding reductions in extracellular concentrations [74, 75]. Lower levels of serotonin have been shown in depressed populations [76], making it a target within treatment interventions. When explored in-vitro, the additions of anti-inflammatory cytokines such as IL-4 and IL-10 have been shown to block the reuptake of serotonin by blocking SERT proteins [77, 78] suggesting a potential treatment option in the treatment of depression.



Allison & Ditor (2014)[14]

Figure 1: Peripheral and central kynurenine pathway interactions

As indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) are found at only very low concentrations within the brain, concentrations of within-brain tryptophan (TRP) and kynurenines are largely dependent on those from the periphery. TRP from the periphery competes with other large neutral amino acids (LNAA) for passage across the blood brain barrier (BBB) to be used in the synthesis of serotonin (5-HT). Kynurenine (KYN) and 3-hydroxykynurenine (3-HK) are also capable of crossing the BBB whereby they participate in the production of kynurenic acid (KYNA) and quinolinic acid (QUIN) which do not easily cross it [14].



Allison & Ditor (2014) [14]

Figure 2: Inflammatory mechanisms of depression

Pro-inflammatory cytokines may contribute to depressive symptoms by means of various mechanisms. (1) Proinflammatory cytokines act on serotonin transporter (SERT) proteins within the brain causing a re-uptake of serotonin (5-HT) and corresponding reduced extracellular concentrations. (2) Proinflammatory cytokines up-regulate enzymes such as indoleamine 2,3- dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) resulting in reduced tryptophan (TRP) availability, ultimately contributing to reduced 5-HT synthesis. (3) Both 3- hydroxykynurenine (3-HK) and quinolinic acid (QUIN) may contribute to elevated levels of reactive oxygen species (ROS) and oxidative stress within the brain. (4) QUIN may induce N- methyl-D-aspartate (NMDA) over-activity thereby contributing to hippocampal atrophy and a loss of glucocorticoid receptors, ultimately leading to a loss of negative feedback and hypothalamic-pituitary-adrenal (HPA) axis over-activity [14].

When exploring the role of inflammation on acute mood states, the administration of an inflammatory pathogen has been shown to increase levels of pro-inflammatory cytokine IL-6, while simultaneously elevating negative mood states [79, 80]. By showing a correlation between inflammation and mood, perhaps reducing inflammation via anti-inflammatory mechanisms will result in an improvement of acute mood states.

2.5.3 Treatments

Current treatments for depression include the use of selective serotonin reuptake inhibitors (SSRI), which work to increase extracellular levels of serotonin by targeting SERT, thus inhibiting their role in serotonin reuptake [74]. The use of SSRIs can be effective in people with major depression, but unfortunately come with a multitude of side-effects in addition to only treating the downstream targets. Although effective in many cases of major depression due to their quick response in alleviating symptoms, SSRIs have been shown to be ineffective in 30% of patients [81]. This concern is compounded by the fact that patients who are resistant to drug therapies tend to demonstrate higher levels of circulating pro-inflammatory cytokines [82]. Treatments that target the immunologic and neurobiological mechanisms, thereby treating the upstream targets are still in the conceptual and pre-clinical stages [83]. Alternative treatments have focused on diet and exercise, as both have shown promise as non-pharmacological treatments for depression, and without the side-effects associated with drug therapy.

Regarding the use of diet, a 12 week anti-inflammatory diet has been shown to be effective in decreasing circulating pro-inflammatory cytokines, which in turn, correlated to decreases in depression and neuropathic pain in the SCI population [84]. Self-reported depression decreased by approximately 40% in that study, with diet-induced decreases in IL-1 β being shown as the mediating factor. Likewise, self-reported neuropathic pain also decreased by approximately 40%, which correlated to diet-induced decreases in IFN- γ and the eicosanoid PGE2.

2.5.4 Exercise as a Treatment Option

Regarding the use of exercise, chronic exercise has been shown to be an effective alternative to drug therapies for people with major depressive disorder (MDD) [21, 85]. Exercise has been shown to be comparably effective in achieving remission in MDD with supervised or home-based exercise programs as common drug therapies [85] and more effective than quiet resting [21]. While a habitual exercise routine should be the goal of any exercise program, a single bout of exercise has also been shown to be effective in decreasing depressive symptoms and improving mood in able-bodied individuals [86–89]. According to the social cognitive approach, depressed mood and depressive disorders such as MDD are considered to be varying intensities of the same phenomena [90]. Thus, the change in mood states might be relevant for depression itself; suggesting that repetitive experiences of mood improvements might affect depressive disorders over long term training studies. The anti-depressant effect of exercise is influenced by an individual's starting point. Larger improvements in depressive mood are seen in those that have a high score for depression or negative mood at baseline [87, 91]. When examining the type of exercise that evokes the best response for improving mood, aerobic exercise is the most consistent in eliciting a positive response. An exercise duration of 10 minutes has been shown to be enough time to improve one's mood, however the majority of literature recommends 20-40 minutes of exercise to evoke the best response [91, 92]. The ideal intensity is also subject to debate, with most studies suggesting that low to moderate intensities are the most effective in improving mood. When exercising at too high of an intensity, negative mood states tend to increase. Therefore, it should be

common practice not to exercise above one's ability level in order to effectively modulate one's mood [91–93].

Acute exercise has also been shown to be effective in special populations for improving mood states [19, 94, 95], and like other populations, aerobic exercise seems to be the best modality. Additionally, the intensity of exercise plays a role in mood alterations, as working at a moderate intensity, as opposed to light and heavy intensities, evoked the greatest positive change in mood [19].

While it has been shown that exercise does indeed alter mood states, the mechanisms behind these changes are currently unknown. There are many hypotheses, all with variable support. When looking at psychological reasons for these changes, researchers argue that increasing self-efficacy and feeling in control of one's own situation is enough to elicit a positive change [90]. Others argue that this change is merely due to exercise acting to avert one's focus away from their depressive thoughts [90]. Further research has explored hormonal, inflammatory, and neurogenesis alterations [96] but no research has explored any potential mechanism and their role in acute mood alteration. It has been shown that there is a decrease in pro-inflammatory cytokines IL-6, IL-8, and TNF- α and increases in the anti-inflammatory cytokine IL-4 in people with MDD after an acute bout of submaximal and maximal exercise [97].

Overall, it has been shown that chronically elevated levels of inflammation can increase the risk of depression, while exercise seems to alleviate depression and depressive mood. Therefore, perhaps exercised-induced decreases in pro-inflammatory, or increases in anti-inflammatory cytokines mediate the exercise-induced decrease in depressive symptoms.

2.6.0 The Effect of Acute Exercise on Cytokines

Regular exercise has consistently shown to be beneficial in preventing all-cause mortality, including cardiovascular disease, type 2 diabetes, obesity and hypertension [98]. Additionally, chronic exercise has been shown to increase anti-inflammatory markers and decrease pro-inflammatory cytokine levels, while a single bout of exercise has consistently shown to have a unique influence on the inflammatory profile [17]. Unlike the inflammatory response that occurs during infection where pro-inflammatory cytokines such as TNF- α and IL-1 β are increased followed by increases in anti-inflammatory cytokines, exercise seems to bypass the initial pro-inflammatory response when working at a moderate intensity. During exercise, the cytokine IL-6 is released from working skeletal muscle, and thus termed a myokine. IL-6, which has roles as both a pro and anti-inflammatory cytokine, increases dramatically with exercise intensity. The increase in circulating IL-6 activates the release of other anti-inflammatory cytokines such as IL-10, IL-1ra, and soluble TNF receptors (sTNFRs) while also inhibiting the pro-inflammatory cytokine TNF- α [17, 99, 100]. Furthermore, with the increase of anti-inflammatory cytokines because of the elevated IL-6 levels, there is additional inhibition of pro-inflammatory cytokines TNF- α , IFN- γ , and IL-1 β . By increasing anti-inflammatory cytokines and decreasing pro-inflammatory cytokines, exercise may play an acute role in changes in both the aforementioned Kynurenine and SERT pathways. Elevations in anti-inflammatory cytokines may be inhibiting the production of pro-inflammatory cytokines, indirectly reducing the activity of IDO [101]. As IDO oxidizes available tryptophan, reducing its activity levels will allow for more tryptophan to become available to be synthesized into serotonin within the brain, thus improving one's

overall mood. Additionally, elevations in anti-inflammatory cytokines will inhibit SERT transporters to further contribute to tryptophan availability. Tryptophan availability is further increased as moderate acute exercise has been shown to increase nonesterified fatty acid (NEFA) mobilization [102]. Albumin, which typically is bound to tryptophan molecules, is displaced by NEFA, as NEFA wants to bind to albumin. This frees up a larger proportion of free form tryptophan that can then be synthesized into serotonin within the brain [102]. These changes are accompanied by a shift in the ratio of kynurenine metabolites, as there is seen to be an increase in the production of the neuroprotective kynurenic acid and a decline in the neurotoxic quinolinic acid peripherally [103, 104].

Mechanisms that may explain these changes include the activation of the HPA axis and increased sympathetic neural output [100]. During exercise, there is an increased release of cortisol from the HPA axis and adrenaline as part of SNS activation, which both precede the rise in IL-6 from the skeletal muscle. These hormones both work to inhibit the release of the pro-inflammatory cytokine TNF- α by monocytes. Cortisol is also augmented by the release of IL-6, further increasing its levels and its regulatory effect in the body. The role of SNS activation during exercise is further supported as individuals with complete tetraplegia, with a blunted sympathetic response, do not have similar anti-inflammatory cytokine increases in circulating blood as those with an intact sympathetic drive [105, 106]. Thus, hormones, myokines, and cytokines all contribute to the anti-inflammatory effect of exercise. Other factors that may influence the exercise-induced change in cytokine levels include the inhibition of macrophage infiltration into adipose tissue, and downregulation of TLRs, which both work to reduce the number of

pro-inflammatory monocytes in the blood. Increased levels of growth hormone, prolactin, and heat shock proteins that influence leukocyte trafficking may also contribute to the anti-inflammatory effect of exercise [99]. When compared to controls, MS and SCI populations not only have higher baseline pro-inflammatory profiles, but also a blunted exercise-induced increase in anti-inflammatory profiles [105–107]. This blunted anti-inflammatory response may be attributed to the aforementioned dysfunction within the endocrine, nervous, and muscular systems.

An alternative option to using basic aerobic exercise is the use of functional electrical stimulation (FES) in a hybrid format with exercise. One study has shown that the combined therapy is more effective in increasing anti-inflammatory markers in a motor complete spinal cord population [108]. While promising for motor complete SCI, the caveat of this type of treatment is that a major limitation for FES is skin sensitivity. For individuals who are hypersensitive to touch, the level of stimulation necessary to evoke a larger anti-inflammatory response cannot be attained. Resistance training has also been explored as a modality for increasing anti-inflammatory levels, however, no changes were found in people with MS [109]. This indicates the role that exercise modality plays in altering inflammation levels, as only aerobic exercise seems to have an influence on increasing anti-inflammatory and decreasing pro-inflammatory markers.

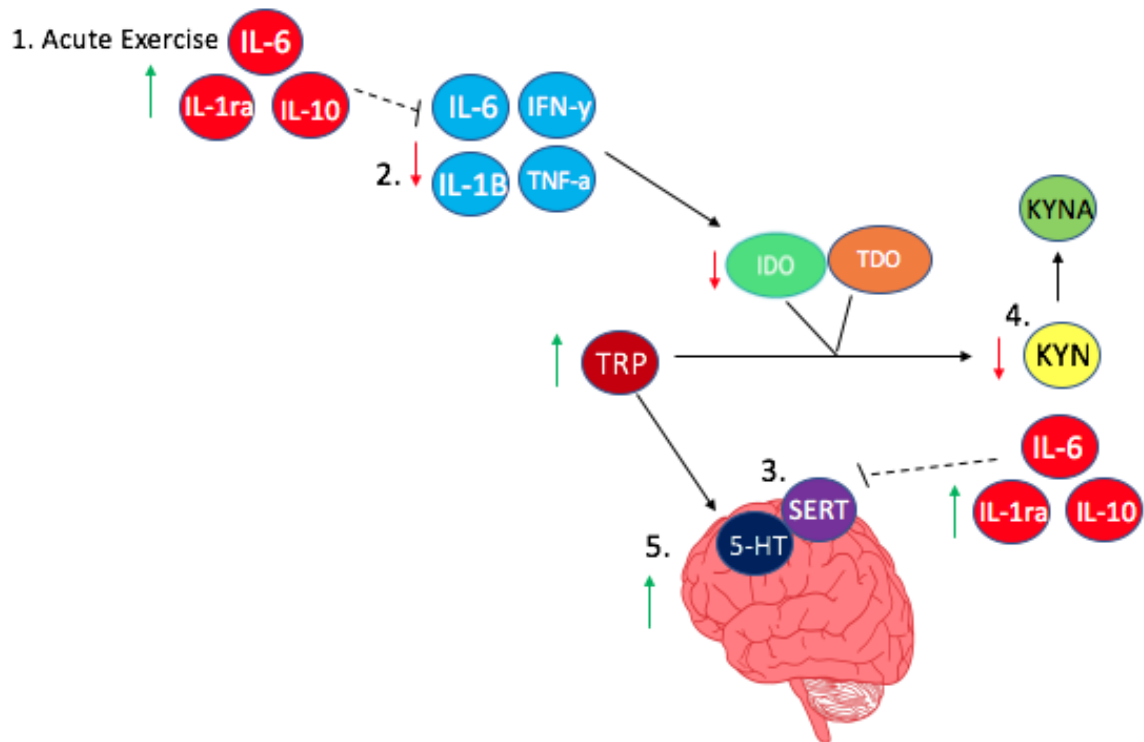


Figure 3: The proposed role of acute exercise on the kynurenine pathway

Acute exercise may increase the release of anti-inflammatory cytokine (1), which work to inhibit both pro-inflammatory cytokines (2) and SERT proteins (3). Inhibiting pro-inflammatory cytokines indirectly reduces the amount of IDO activity and thus TRP availability. Kynurenine (4) will also see a shift in its metabolite ratio, with an increase in the ratio of the neuroprotective kynurenic acid (KYNA) instead of the neurotoxic QUIN. Additionally, anti-inflammatory cytokines reduce SERT proteins, therefore decreasing 5-HT reuptake and resulting in more available TRP. With more TRP available to be synthesized into 5-HT in the brain, there should be a corresponding improvement in mood (5).

2.7.0 Conclusion

In conclusion, people with multiple sclerosis and spinal cord injuries have chronically elevated basal levels of pro-inflammatory markers. This contributes to an elevated risk of secondary health complications such as depression that further increases pro-inflammatory markers, creating a vicious cycle. As an alternative of using drug therapies, as they have been shown to have limitations, the utilization of moderate, acute exercise has been shown to both improve mood and alter cytokine profiles for a short period of time, but have never been examined together. By examining the potential link between these changes in inflammatory markers and alterations in mood, this study offers further evidence to the benefits of moderate exercise in combatting secondary health problems in special populations in addition to contributing a potential mechanism for exercise-invoked mood changes.

III. Purpose & Hypotheses

3.1.0 *Statement of Purpose*

The purpose of this study is to determine if the commonly observed acute exercise-induced changes in mood are related to exercise-induced changes in cytokines, in individuals with MS and SCI.

3.2.0 *Hypothesis*

Previous work in this lab has shown that improvements in mood via an anti-inflammatory diet occurs after just 1 month. Further, pilot work that preceded this thesis showed that an acute bout of exercise at a moderate intensity in MS and SCI populations was effective in improving their mood by decreasing negative mood states (Appendix F). Therefore, our hypothesis is that a single bout of moderate intensity exercise will induce positive changes in mood in individuals with MS and SCI. Additionally, we hypothesize that there will be an exercise-induced increase in anti-inflammatory cytokines and a decrease in pro-inflammatory cytokines, and that these changes in inflammation will mediate the exercise-induced changes in mood via the Kynurenine pathway.

IV. Overview of Study Design

4.1.0 Study Design and Participants

Participant recruitment occurred between January and April 2017. The study consisted of two exercise sessions separated by a minimum of five days. Participants with various levels and severities of SCI and MS were recruited for participation in the study from the Brock-Niagara Centre for Health and Well-Being Power Cord exercise program. Inclusion criteria included (1) over the age of 18, (2) SCI of any level or severity (American Spinal Cord Injury Association A-D) or (3) MS types of relapse-remitting or secondary progressive with an EDSS between 3-8 (Kurtzke Expanded Disability Status Score), and (4) at least 1-year post injury/diagnosis. Exclusion criteria included (1) being unable to fill out the questionnaires independently, (2) unstable medical condition within 2 weeks prior to testing, and (3) currently taking anti-inflammatory medication or anti-depressants. Participant characteristics are shown in Table 1. Thirteen individuals [3 male, 10 female, age 57.2 ± 7.6 years with chronic [4-42 years] MS [relapse-remitting or secondary progressive; EDSS 3.0-6.0] or with chronic [1-24years] SCI [C2-L2; AIS A-D]] were recruited for participation in the study. The study received ethical approval from the Brock University Research Ethics Board (File No. 16-073 - DITOR). All data was collected on-site at Brock University and the Brock-Niagara Center for Health and Well-Being.

Table #1: Participant Characteristics

MS/SCI	TYPE	EDSS/ AISA	SEX	AGE (YEARS)	YEARS POST (YEARS)	RELATIVE VO ₂ PEAK (ml/kg/min)
MS	SPMS	6.0	F	57	25	19.4
MS	RRMS	3.5	F	42	9	29.5
MS	SPMS	6.0	F	59	31	26.5
MS	SPMS	6.0	F	60	42	23.6
MS	RRMS	4.0	F	46	4	17.1
MS	SPMS	6.0	M	57	23	22.3
MS	RRMS	3.0	F	68	17	26.9
SCI	C2	D	M	65	1	27.8
SCI	T3	D	F	57	1	15.0
SCI	T4	A	F	62	24	-
SCI	C7	A	F	48	10	13.9
SCI	L2	D	F	63	5	31.8
SCI	T6	A	M	60	3	35.0
AVE				57.2	15.0	24.1
SD				7.6	13.1	6.7

4.2.0 Exercise ProtocolVisit # 1

Participants were asked to come in in a fasted state, having abstained from caffeine and heavy exercise for the last 24 hours, prior to completing a VO₂ peak test on a recumbent hybrid arm-leg ergometer (Nustep T5 Recumbent Cross Trainer; Ann Arbor, Michigan). Participants underwent a 5-minute warm-up at 0 resistance. Following the warm-up, a sealed mask, which covered their mouth and nose was secured on their face for the duration of the protocol. The mask measured inspired O₂ levels and expired CO₂

levels every 30 seconds using indirect calorimetry (VacuMed® Vista Mini-CPX metabolic cart, Ventura, CA). The test began at a low resistance, relative to a self-selected ability level, and increased in resistance by one level every 2 minutes. Participants were asked to maintain a speed of 100 strides per minute (spm) for the duration of the protocol. The test was considered over when either the participant voluntarily quit, their cadence dipped below 80 spm for 3 seconds, or there was a plateau seen in the O₂ levels inspired with increasing workloads. While undergoing the test protocol, the workload measured in watts (W) was recorded. The highest recorded inspired O₂ level over a 30s-collection period was considered the participant's VO₂ peak. The wattage obtained during time point was used to calculate the workload of 60% VO₂ for their second session. After completing this protocol, participants completed a 5-minute cool down at 0 resistance.

Visit # 2

At least five days after completing session # 1, participants underwent 30 minutes of exercise at 60% of their previously determined VO₂ peak on the Nustep. Prior to exercise, a nurse inserted an indwelling catheter into the antecubital vein of the participant's non-dominant arm. A total of 15mL of blood was collected into three BD vacutainer serum collection tubes at baseline, which were immediately placed on ice and allowed to clot for 30 minutes prior to centrifugation. Participants were also asked to complete the 65-item Profile of Mood States (POMS) questionnaire. After a light 5-minute warm-up on the NuStep, participants were asked to workout at 60% VO₂ peak for 30 minutes. To ensure they were working at a moderate intensity they were asked every 2 minutes how hard they felt they are working with a 6-20 Borg Rate of Perceived Exertion

(RPE) Scale to ensure they did not exceed a score of 13, which is designated as “somewhat hard” and representative of 60% VO₂ peak workload. The resistance level was altered accordingly to ensure they maintained a 13 RPE. Upon completion of the 30 minutes of exercise, participants underwent a 20mL blood draw (the first 5 mL was discarded as per indwelling catheter guidelines) which was then placed on ice and allowed to clot for 30 minutes prior to centrifugation. Participants were then asked to complete a second POMS questionnaire. Participants were then placed in a private location, to avoid outside influences that may affect mood, for one hour of quiet rest after which the final blood draw of 20mL (first 5mL discarded) and a final POMS questionnaire was completed.

Upon completion of the exercise protocol, the blood samples were allowed to clot for 30 minutes followed by centrifugation at a speed of 1000 times gravity, in a temperature controlled centrifuge (4°C) for a duration of 15 minutes. Serum was separated and aliquoted at a volume of 0.5ml into individual Eppendorf tubes (to avoid thaw, re-freeze cycles) and frozen at -80° C until later analysis.

4.3.0 Quantification of serum inflammatory markers and amino acids

Upon completion of the exercise protocol by all the participants, serum from each of the three-time points were thawed and assayed together on the same plate.

Inflammatory mediators of interest included: pro-inflammatory cytokines TNF- α , IFN- γ , and IL-6; anti-inflammatory cytokines IL-6; and the amino acids tryptophan (TRP), and kynurenine (KYN). Analysis of pro and anti-inflammatory cytokines was performed in triplicate via the Magpix Multiplex system (EMD Millipore, MA, U.S.A) and analyzed

using Luminex software. Analysis of amino acids was performed in triplicate using enzyme-linked immunosorbent assay (Immunodiagnostik, Bensheim, Germany; Labor Diagnostika, Nordhorn, Germany) Ratios for KYN/TRP was used to estimate the activity level of the enzyme indoleamine 2,3-dioxygenase (IDO).

4.4.0 Assessment of Mood

Participants were asked to complete the POMS questionnaire at each time point as a measure of their current mood. The POMS is a self-administered, 65-item questionnaire that assesses participants on six separate sub scales: tension, depression, anger, vigor, fatigue, and confusion [110]. Participants rated each item on a 5-point Likert scale where 1 indicates “Not at all”, and 5 denotes a score of “Extremely.” Beginning with a base score of 20 to avoid any negative values, the negative subscale scores (tension, depression, anger, fatigue, and confusion) are added together and subtracted by the vigour score to form an overall measure of affect that is labeled total mood disturbance. The POMS has been validated as an effective method to measure acute mood changes [111].

4.5 Assessment of Inflammatory Markers

Inflammatory mediators of interest specific to depressed mood and chronic inflammation included the pro-inflammatory cytokines: IL-6, TNF- α , and IFN- γ [10]. These cytokines are known to stimulate the activity of the enzyme IDO [6]. The activity of IDO is of interest due its role in the breakdown of TRP into the metabolite kynurenine. As TRP plays a vital role as the precursor for serotonin synthesis, it was important to measure pre and post exercise levels.

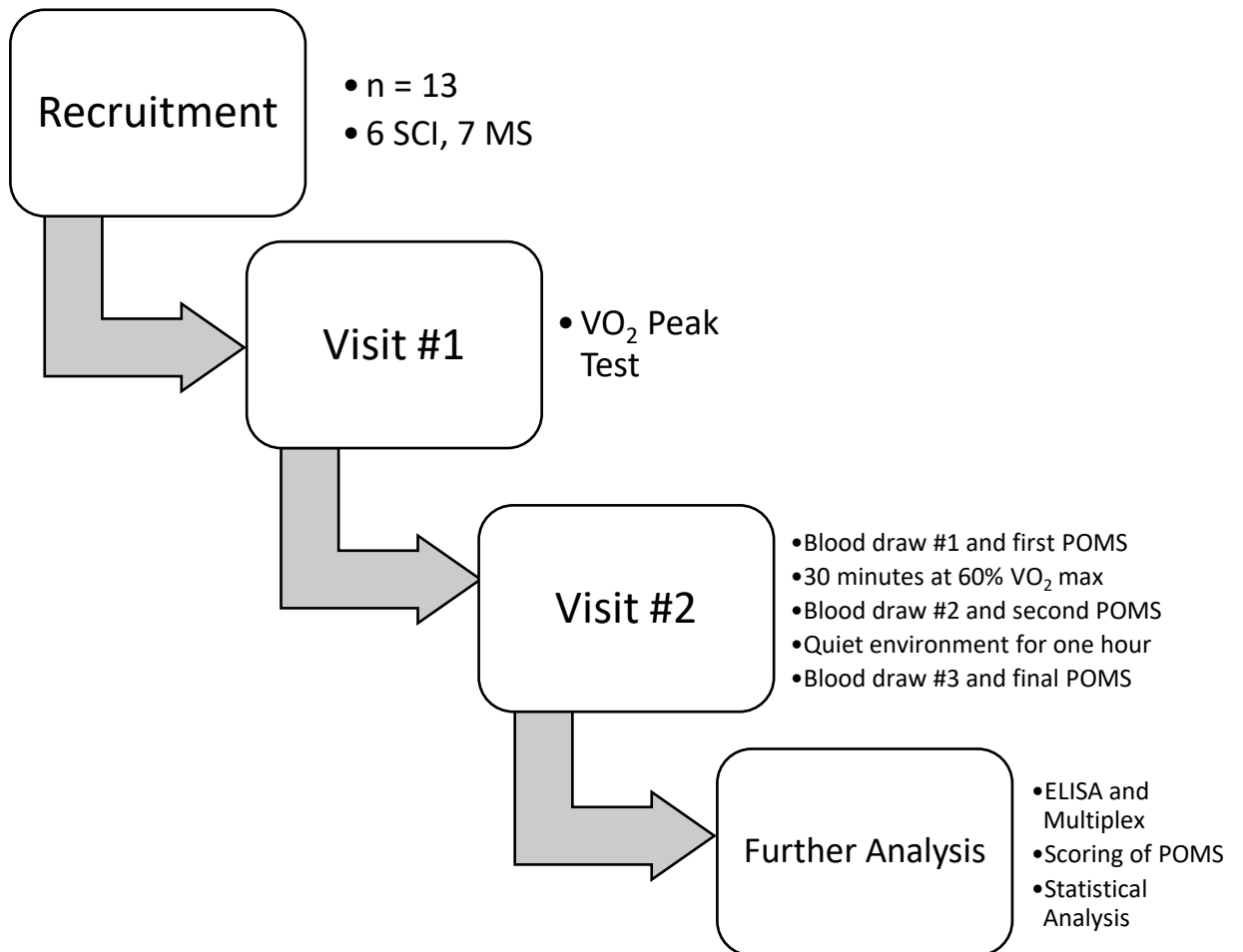
Exercise has been shown to increase levels of anti-inflammatory cytokines such as IL-6 [17]. As anti-inflammatory cytokines inhibit the production of the pro-inflammatory cytokines of interest, they may therefore indirectly affect the quantity and activity of IDO, TRP degradation, and corresponding metabolites.

4.6 Statistical Analysis

Normality was determined using the Kolmogorov-Smirnov test of normality with Lilliefors significance correction. A one-way repeated measures ANOVA (3 levels for time; baseline, immediately post, and one-hour post exercise) for blood markers of interest and POMS scores was performed for normally distributed data. If the data was not normally distributed, non-parametric analyses was performed. A Friedman's test of differences among repeated measures (baseline, immediately post, 1-hour post exercise) was performed. If the Friedman's test resulted in a significant value, a Wilcoxon signed-rank test was performed to determine which time points were significantly different from one another.

Finally, a Pearson's r correlation analysis was completed to assess the relationship between the exercise-induced changes in cytokine markers and changes in the POMS questionnaire. For non-normally distributed data, a Spearman's rank order correlation was performed. Statistical significance was set at $p \leq 0.05$ for all tests.

Figure 4: Protocol Flowchart



V. Results

5.1.0 Exercise Adherence

All subjects successfully completed both exercise protocols in their entirety without any adverse events. Due to transportation issues, one participant was unable to complete the one-hour post-exercise measurements. Their data was thus only used for analysis in pre to post exercise changes for all measures.

5.2.0 Changes in POMS

Changes in Profile of Mood States (POMS) scores are shown in Figure 5. Normality was determined using the Kolmogorov-Smirnov test with Lillifors significance correction. The Friedman test showed that there was a statistically significant reduction in total mood disorder (TMD) across time points (chi-square= 8.55, $p=.01$). Post hoc analysis performed with the Wilcoxon signed-rank test showed significant reductions from both pre to post and pre to one hour post ($z=-2.239$, $p=.03$; $z=-2.669$, $p=.01$). When analyzed separately, the Friedman test showed that there was a statistically significant reduction across the time points in the sub-scores tension, depression, and anger (chi-square=8.93, $p=.01$; chi-square=9.25, $p=.01$; chi-square=7.03, $p=.03$). Post hoc analysis performed with the Wilcoxon signed-rank test showed significant reductions in tension pre to post and pre to one hour post ($z=-2.139$, $p=.03$; $z=-2.407$, $p=.02$); significant reductions in depression pre to post ($z=-2.036$, $p=.04$); and significant reductions in anger pre to post and pre to one hour post ($z=-2.375$, $p=.02$; $z=-2.025$, $p=.04$). The Friedman test showed that there was no significant change in fatigue across time. One-way repeated measures ANOVA were performed for normally distributed sub-scores confusion and vigour and showed a statistically significant decline in confusion over the time points

($f=5.06$, $p=.03$). Post hoc analysis performed using Bonferroni test for multiple comparisons showed no statically significant difference between time points, however, there was a trend for the reduction of confusion between pre to one hour post ($\theta=-2.25$, $p=.06$).

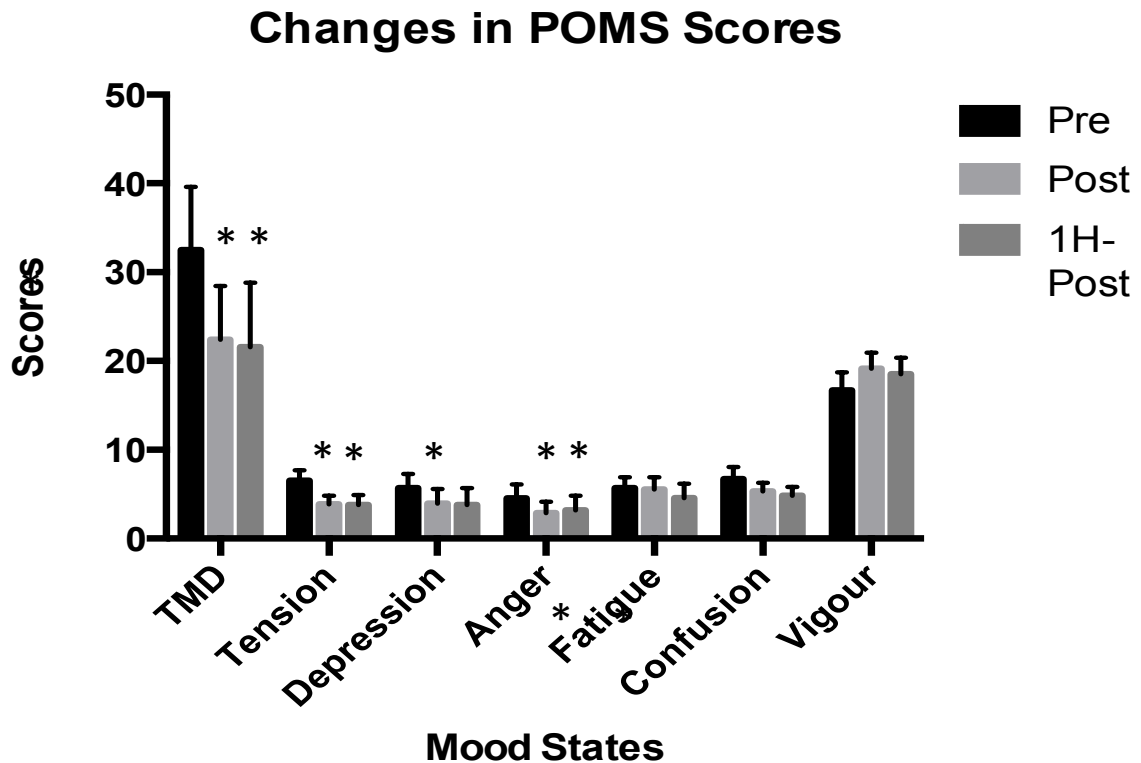


Figure 5: Change in POMS Scores across Time points

5.3.0 Changes in Inflammatory Mediators

Changes in serum inflammatory mediators are shown in Table 2. One-way repeated measures ANOVA were used to measure changes in IL-6, TNF- α , and IFN- γ across time points. There was a significant reduction in TNF- α ($f=18.84$, $p=.01$) and a trend for the decrease of IFN- γ ($f=3.68$, $p=.06$). Further post hoc analysis with Bonferroni

test for multiple comparisons showed a significant reduction of TNF- α values between pre to one hour post ($\theta=-3.86$, $p=.001$) and post to one hour post ($\theta=-2.82$, $p=.016$). The analysis of the serum anti-inflammatory cytokine IL-10 was attempted, but unfortunately did not work due to technical difficulties.

Table #2: Change in Inflammatory Mediators

	Pre	Post	1 H Post	1-Way ANOVA (p-value)	Pre- Post	Pre- 1H Post	Post- 1H Post
IL-6	10.13 \pm 3.31	9.51 \pm 3.03	9.91 \pm 2.91	.325			
TNF- α	25.97 \pm 8.3	24.93 \pm 7.91	23.17 \pm 6.94	.01*	.151	.001*	.016*
IFN- γ	40.7 \pm 13.19	37.63 \pm 11.07	38.74 \pm 11.73	.06	.227	.155	1.00

*All inflammatory mediators are displayed in pg/ml. All results are shown as mean \pm SD. All statistical analysis scores are represented as p-values. *Significantly different with $P<0.05$*

5.4.0 Change in Amino Acids

Values for serum amino acids are show in Table 3. The Friedman test showed no statistically significant change in kynurenine or the ratio of kynurenine to tryptophan to estimate IDO activity (chi-square=0.0 $p=.74$; chi-square=2.36, $p=.307$). One-way repeated measures ANOVA were used to measure changes in across time points for tryptophan. There was no statistically significant change ($f=2.25$, $p=.13$; $f=0.36$).

Table #3: Change in Amino Acids

	Pre	Post	1 H Post	1-Way ANOVA (p-value)	Friedmans (p-value)	Pre- Post	Pre- 1H Post	Post- 1H Post
KYN	3.02±1.33	3.50±2.64	2.69±0.86		.735			
TRP	99.93±22.91	87.62±24.20	99.28±26.83	.130		.086	1.00	.516
KYN/TRP	33.05±11.55	25.04±18.14	36.92±8.25		.307			

*All amino acids are displayed in $\mu\text{mol/L}$. All results are shown as mean \pm SD. All statistical analysis scores are represented as p-values. KYN: Kynurenine; TRP: Tryptophan; KYNA: Kynurenic acid. *Significantly different with $P < 0.05$*

5.5.0 Correlations between POMS and Blood Markers

Correlations between the changes in POMS scores and the changes in blood markers pre to post and pre to one hour post are shown in Tables 4 and 5. If the data was normally distributed, a Pearson's r correlation was conducted. If the data was not normally distributed, a nonparametric Spearman correlation was performed. Significant changes were shown from pre to post changes in TNF- α and changes in pre to post vigour ($r = -.577$ $p = .049$). There was a significant relationship shown between changes in pre to one hour post scores of IFN- γ and changes in fatigue ($r = -.633$ $p = .036$). There was also a trend between changes in IFN- γ and tension sub-scores ($r = .528$ $p = .095$) and changes in TNF- α with changes in tension and depression sub-scores ($r = .551$, $p = .099$; $r = .622$, $p = .055$). Significant relationships were shown between pre to post changes in tryptophan and pre to post changes in depression ($r = .676$ $p = .011$) as well as between pre to post changes in tryptophan and pre to post changes in vigour ($r = -.577$, $p = 0.48$); there were trends for changes in pre to post tryptophan with pre to post changes in TMD, tension, and anger sub-scores ($r = .519$ $p = .069$, $r = .491$ $p = .089$, $r = .481$ $p = .096$). A trend

was also shown in the changing ratio of kynurenine and tryptophan to the changes in pre to one hour post depression ($r=.505$ $p=.094$).

Table #4: Pre to Post Exercise Correlations

	Δ TMD	Δ Tension	Δ Depression	Δ Anger	Δ Fatigue	Δ Confusion	Δ Vigour
Δ KYN	.074 ^b	-.068 ^b	.345 ^b	0.00 ^b	.112 ^b	-.329 ^b	.171 ^b
Δ TRP	.519 ^a	.491 ^a	.676 ^{a*}	.531 ^b	-.084 ^a	-.055 ^b	-.557 ^{a*}
Δ KYN/TRP	.292 ^a	.360 ^a	.023 ^a	.112 ^b	.123 ^a	.370 ^b	-.306 ^a
Δ IL-6	.081 ^a	.195 ^a	.350 ^a	.081 ^b	-.209 ^a	-.053 ^b	-.044 ^a
Δ TNF- α	.379 ^a	.160 ^a	.369 ^a	-.004 ^b	-.033 ^a	.167 ^b	-.577 ^{a*}
Δ IFN- γ	-.162 ^b	-.074 ^b	-.376 ^b	-.150 ^b	-.018 ^b	.371 ^b	.057 ^b

*All correlations are represented as r-values. ^a represents Pearson Correlation. ^b represents Spearman's Correlation. *Significantly correlated at $P<0.05$*

Table #5: Pre to 1 Hour Post Exercise Correlations

	Δ TMD	Δ Tension	Δ Depression	Δ Anger	Δ Fatigue	Δ Confusion	Δ Vigour
Δ KYN	-.442 ^b	-.312 ^b	-.139 ^b	-.212 ^b	-.326 ^b	-.128 ^b	.282 ^b
Δ TRP	.208 ^a	.173 ^a	.728 ^{b*}	.031 ^a	-.039 ^a	.256 ^a	.091 ^a
Δ KYN/TRP	.495 ^b	.453 ^b	.505 ^b	.286 ^b	.196 ^b	.307 ^b	-.218 ^b
Δ IL-6	.167 ^a	.323 ^a	.287 ^a	.008 ^a	-.149 ^a	.245 ^a	.072 ^a
Δ TNF- α	.409 ^a	.551 ^a	.622 ^a	-.248 ^a	-.010 ^a	.431 ^a	-.076 ^a
Δ IFN- γ	-.087 ^b	.528 ^b	-.166 ^b	-.069 ^b	-.633 ^{b*}	-.189 ^b	-.077 ^b

*All correlations are represented as r-values. ^a represents Pearson Correlation. ^b represents Spearman's Correlation. *Significantly correlated at $P < 0.05$*

5.6.0 Subgroup Analysis

Multiple participants scored a 0 on the depression sub-score of the POMS at baseline, and therefore do not have the ability to improve their mood. Further analyses were completed by taking a subgroup of participants who were shown to have a positive response to the acute bout of exercise in order to further determine if changes in mood scores had a relationship with the changes in blood markers. This subgroup was described as participants who decreased their depression sub-score by at least 2 points pre to post exercise as most participants had either no change in depression scores or changes of at least two. The resulting analysis reduced the sample size to 7 participants.

5.6.1 Subgroup Changes in POMS

Changes in the subgroup's Profile of Mood States (POMS) scores are shown in Figure 6. A one-way repeated measures ANOVA showed that there was a statistically significant reduction in TMD across time points ($f= 18.53$, $p=.001$). Post hoc analysis performed using Bonferroni test for multiple comparisons showed that there was a significant difference in the reduction of TMD between pre and post exercise ($\theta=-18.86$, $p=.001$) and pre and 1 hour post exercise ($\theta=-18.14$, $p=.021$). When TMD was separated into its sub scores, one-way repeated measures ANOVAs showed a significant difference between time points for tension and depression ($f= 8.52$, $p=.02$; $f=19.12$, $p=.002$) and a trend for vigour ($f= 3.92$, $p=.077$). Post hoc analysis performed using Bonferroni test for multiple comparisons showed that there was a significant difference for tension pre to post exercise ($\theta=-4.71$, $p=.017$), depression pre to post and pre to 1 hour post exercise time points ($\theta=-3.7$, $p=.001$; $\theta=-5.00$, $p=.01$). Friedman's test for non-parametric data showed there was a significant reduction in anger and confusion across time points (chi-square= 7.14, $p=.028$; chi-square= 7.76, $p=.021$). Post hoc analysis performed with the Wilcoxon signed-rank test showed significant reduction pre to post exercise in anger and confusion ($z=-2.21$, $p=.027$; $z=-2.23$, $p=.021$) and a trend for pre to 1 hour post exercise in both measures ($z=-1.80$, $p=.072$; $z=-1.95$, $p=.051$). There was also a significant reduction in fatigue from pre to 1 hour post exercise ($z=-2.00$, $p=.046$).

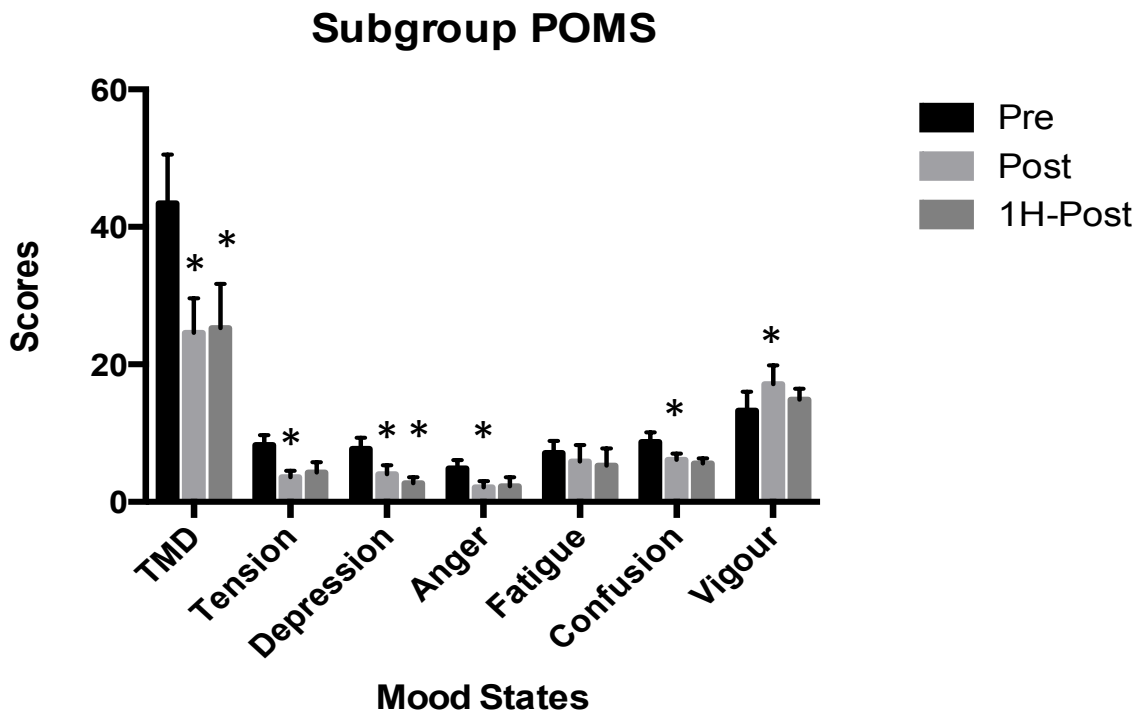


Figure #6: Subgroup Change in POMS Scores across Time points

5.6.2 Subgroup Changes in Inflammatory Mediators

Changes in serum inflammatory mediators are shown in Table 6. One-way repeated measures ANOVA were used to measure changes in IL-6, TNF- α , and IFN- γ across time points. There was a significant difference in TNF- α ($f=14.8$, $p=.004$). Post hoc analysis performed using Bonferroni test for multiple comparisons showed a significant reduction of TNF- α values between pre to post ($\theta=-1.38$, $p=.043$) and pre to one hour post exercise ($\theta=-4.14$, $p=.012$). There was also a trend in the reduction of TNF- α from post to 1 hour post exercise ($\theta=-2.76$, $p=0.63$). There were no significant changes in IL-6 ($f=1.14$, $p=.343$) or IFN- γ ($f=.92$, $p=.395$) across time points.

Table #6: Subgroup Change in Inflammatory Mediators

	Pre	Post	1 H Post	1-Way ANOVA (p-value)	Pre- Post	Pre- 1H Post	Post- 1H Post
IL-6	11.14±3.89	10.10±3.76	11.23±2.93	.343			
TNF- α	29.04±6.84	27.66±6.01	24.89±6.68	.004*	.043*	.012*	.063
IFN- γ	46.35±16.14	43.13±12.44	43.60±12.69	.395			

*All inflammatory mediators are displayed in pg/ml. All results are shown as mean \pm SD. All statistical analysis scores are represented as p-values. *Significantly different with $P < 0.05$*

5.6.3 Subgroup Change in Amino Acids

Changes in serum amino acids are shown in Table 7. A one-way repeated measures ANOVA showed a significant reduction in tryptophan ($f=4.87$, $p=.028$). Post hoc analysis performed using Bonferroni test for multiple comparisons showed a significant difference in the reduction of tryptophan from pre to post exercise ($\theta=-19.47$, $p=.031$). No statistically significant changes were found for kynurenine ($f=1.23$, $p=.333$) or the kynurenine to tryptophan ratio across time points ($\chi^2=1.33$, $p=.513$).

Table #7: Subgroup Change in Amino Acids

	Pre	Post	1 H Post	1-Way ANOVA (p-value)	Friedmans (p-value)	Pre- Post	Pre- 1H Post	Post- 1H Post
KYN	2.62±1.02	2.08±0.68	2.73±1.02	.333				
TRP	106.06±28.36	86.59±28.08	94.02±26.95	.028*		.031*	.281	1.00
KYN/TRP	42.67±9.52	43.47±16.75	36.53±8.07		.513			

*All amino acids are displayed in $\mu\text{mol/L}$. All results are shown as mean \pm SD. All statistical analysis scores are represented as p-values. KYN: Kynurenine; TRP: Tryptophan; KYNA: Kynurenic acid. *Significantly different with $P < 0.05$*

5.6.4 Subgroup Correlations between POMS and Blood Markers

Correlations between the changes in POMS scores and the changes in blood markers pre to post and pre to one hour post are shown in Tables 8 and 9. Normally distributed data was assessed via Pearson's r , while non normally distributed data was assessed via a nonparametric Spearman correlation. Significant relationships were shown between pre to post changes in IFN- γ and changes in confusion ($r=.883$, $p=.02$) as well as pre to post changes in TNF- α and changes in vigour ($r=-.760$, $p=.047$). While exploring possible correlations between the changes between pre and 1 hour post exercise, there was a significant relationship between the change in IFN- γ and change in tension ($r=.943$, $p=.005$); change in depression and IL-6 ($r=.853$, $p=.031$); and change in IFN- γ and change in fatigue ($r=-.841$, $p=.036$). There was also a trend found for the correlation between the change in TNF- α and change in depression sub-scores ($r=.722$, $p=.067$). When looking at the correlation between mood changes and changes in amino acids multiple trends were shown. Trends were shown for a correlation between changes in TMD with changes in kynurenine and changes in the ratio of kynurenine to tryptophan

($r=-.783$, $p=.066$; $r=.764$, $p=.077$). There were also trends for the correlation between changes in depression and kynurenine and changes in depression with the changes of the ratio of kynurenine to tryptophan ($r=-.772$, $p=.072$; $r=.736$, $p=.096$). Trends were seen for changes in anger and tryptophan, and changes in confusion and kynurenine ($r=.733$, $p=.061$; $r=-.741$, $p=.092$). Finally, there was a trend seen between the changes pre to 1 hour post in TNF- α and depression ($r=.722$, $p=0.67$).

Table #8: Subgroup Pre to Post Exercise Change Correlations

	Δ TMD	Δ Tension	Δ Depression	Δ Anger	Δ Fatigue	Δ Confusion	Δ Vigour
Δ KYN	-.783 ^b	-.698 ^b	-.772 ^b	0.00 ^b	-.029 ^b	-.741 ^b	.647 ^b
Δ TRP	.374 ^a	.172 ^a	.576 ^a	.733 ^a	-.205 ^a	-.132 ^a	-.408 ^a
Δ KYN/TRP	.764 ^a	.697 ^a	.736 ^a	.085 ^a	.194 ^a	.399 ^a	-.315 ^a
Δ IL-6	-.668 ^a	.285 ^a	-.166 ^a	-.406 ^a	-.595 ^a	-.310 ^a	-.013 ^a
Δ TNF- α	.193 ^a	.322 ^a	-.189 ^a	.052 ^a	-.447 ^a	.334 ^a	-.760 ^{a*}
Δ IFN- γ	.696 ^b	.290 ^b	0.00 ^b	-.203 ^b	.257 ^b	.883 ^{b*}	-.395 ^b

*All correlations are represented as r-values. ^a represents Pearson Correlation. ^b represents Spearman's Correlation. *Significantly different with $P<0.05$.*

Table #9: Subgroup Pre to 1 Hour Post Exercise Change Correlations

	Δ TMD	Δ Tension	Δ Depression	Δ Anger	Δ Fatigue	Δ Confusion	Δ Vigour
Δ KYN	-.414 ^b	-.018 ^b	.543 ^b	-.090 ^b	-.559 ^b	-.214 ^b	.523 ^b
Δ TRP	-.090 ^b	.523 ^b	.543 ^b	-.414 ^b	-.577 ^b	-.036 ^b	-.018 ^b
Δ KYN/TRP	.144 ^b	.288 ^b	.075 ^b	.198 ^b	.216 ^b	-.107 ^b	-.342 ^b
Δ IL-6	.429 ^a	.595 ^a	.853 ^{a*}	.059 ^a	-.239 ^a	.273 ^a	-.053 ^a
Δ TNF- α	.435 ^a	.636 ^a	.722 ^a	-.400 ^a	-.095 ^a	.403 ^a	-.204 ^a
Δ IFN- γ	.029 ^b	.943 ^{b*}	.647 ^b	-.029 ^b	-.841 ^{b*}	-.200 ^b	.143 ^b

*All correlations are represented as r-values. ^a represents Pearson Correlation. ^b represents Spearman's Correlation. *Significantly different with $P < 0.05$*

VI. Discussion

6.1.0 Major Findings

The current study was the first to examine the potential effects of acute exercise on mood and the role of cytokines as a mechanism underlying these mood changes in people with multiple sclerosis and spinal cord injury. The main finding from this study was that 30 minutes of aerobic exercise at 60% of VO_2 peak is an effective means to improve mood in these populations. While attempting to explore a potential mechanism that may explain the improvements shown in mood, correlations between mood and blood markers were conducted for both the entire study population as well as in a sub-group of those whose depression sub-score dropped by a minimum of 2 points with exercise. Due to the inconsistent correlations shown for the main group and the sub group, this study suggests that cytokines are probably too slow to affect mood changes immediately, but may instead play a limited role in maintaining the improved mood over a longer duration. This is suggested by the lack of correlations between changes in cytokines and changes in mood from pre to post exercise, but instead correlations appearing between pre to one hour post exercise. However, results from this study should be taken with caution due to its small sample size. Additionally, cytokines may not be acting through the Kynurenine Pathway, but instead through other mechanisms beyond the scope of this study.

When exploring the changes in mood, total mood disturbance was shown to decline ($z=-2.239$, $p=.03$), indicating an improvement in mood, and the improved mood was maintained over an hour of quiet rest. Contributing to the improvement in mood, was the decline in tension, depression, and anger sub-scores from pre to post exercise time points ($z=-2.239$, $p=.03$; $z=-2.036$, $p=.04$; $z=-2.375$, $p=.02$), and these changes were maintained one hour post exercise. The overall exercise-induced mood enhancement that was suggested in this study is consistent with the literature in both able-bodied [92, 111, 112] and special populations [19–21, 93, 113] at a moderate intensity. The results from the POMS questionnaire in the current study also corroborate findings in depressed populations from Bartholomew et al. (2005) and Meyer et al. (2016). Ensari et al (2016) had people with MS exercise at different intensities and saw general reductions in the negative sub-scores, but pre-scores began much lower than normal values, making it

difficult to produce any significant findings due to their lower starting point. Similarly, when participants began at a higher negative score, there was a greater improvement in mood as a result of acute exercise.

Upon initial analysis, the change in tryptophan appeared to have a role in the change in mood as both significant correlations and trends for many of the mood sub-scores pre to post exercise were found. However, all relationships except for the trend between the change in tryptophan and change in anger sub-score were lost in the subgroup analysis. A significant correlation that remained in both groups was the negative correlation between TNF- α and vigour, where a decrease in TNF- α led to an increase in vigour. There are no known studies that have linked TNF- α with vigour, however high levels of TNF- α are generally seen in depressed populations [70, 83], who are shown to also have lower levels of vigour [86] which may explain this relationship. The subgroup also showed trends for positive correlations between the ratio of kynurenine to tryptophan, an indication of IDO activity, and changes in TMD and depression indicating that higher IDO activity is associated with a higher TMD and depression score, which is consistent with the literature [67]. Finally, there was a significant positive correlation between changes in IFN- γ and confusion, which is consistent with one other study that has made a link between delirium and high levels of IFN- γ [114].

Of the blood markers of interest, tryptophan and TNF- α were the only ones to significantly decrease with exercise in the subgroup of participants whose depression sub-score decreased ($z=-19.47$, $p=.031$; $z=-1.38$, $p=.043$). Additionally, TNF- α further decreased from pre to 1 hour post exercise ($z=-4.15$, $p=.012$). The reduction in total tryptophan levels are comparable to the decline in tryptophan levels that have been seen in high intensity exercise [104, 115], however, it has also been shown that free tryptophan, or tryptophan that is no longer bound to albumin, does indeed increase with moderate exercise [102, 116, 117], which is a greater indicator for tryptophan's ability to be synthesized into serotonin. Competition for transport across the BBB with other large neutral amino acids (LNAA) may also influence the amount of serotonin that is able to be synthesized in the brain [102, 117]. Calculating the changing ratio between TRP and LNAA allows for the estimation of tryptophan being transferred into the brain. The

reduction in TNF- α is consistent with moderate intensity exercise studies with values either declining or being maintained [105, 107, 118, 119]; high intensity exercise that may cause muscle damage has been shown to increase levels of TNF- α [18, 97, 99].

The fact that IL-6 levels did not change with exercise goes against the majority of exercise literature, with expected increases ranging from a two to 100-fold increase depending on exercise intensity [99, 120]. It is generally seen even in MS and SCI populations [9, 107], albeit with smaller increases compared to a healthy population. The lack of response in this study may be due to the combined effect of cytokine blunting in the special populations in addition to the intensity of the exercise protocol not being sufficient to alter cytokine profiles [121, 122]. The lack of IL-6 response may also explain the lack of changes in other inflammatory blood markers, as IL-6 is known to be a precursor to alterations in other cytokines and amino acids downstream [17].

Pre to one hour post correlations exhibited more consistent relationships between changes in cytokines and changes in mood scores, as a significant positive correlation was found between IL-6 and depression in the sub-group, as well as positive trends in both groups for changes in TNF- α and depression. Other significant negative correlations that were found were between IFN- γ and tension and fatigue sub-scores, with IFN- γ and fatigue correlations significant for both groups, which has previously been found in MS populations [123].

6.2.0 Clinical/ Physiological Significance

This study suggests that a single bout of moderate intensity exercise for 30 minutes is sufficient to improve the mood of people living with multiple sclerosis and spinal cord injuries and this improved mood may be maintained for at least an hour. By successfully improving someone's mood after just a single bout, exercise can be used as a prescription to reduce negative mood symptoms for a short period of time. An accumulative effect of multiple bouts of exercise may then be attainable to decrease not just negative and depressive mood, but as a treatment consideration for people who are depressed. The fact that both populations are 2-10 times more likely to suffer from depression signifies the importance of finding alternative methods to treating depressed mood before it develops into depression. A single bout of moderate intensity exercise is a

holistic option to be paired with drug therapies that may be prescribed, contributing to mounting evidence on the many benefits of exercise.

This thesis aimed to explore the potential mechanisms behind the commonly observed exercise-induced changes in mood in chronically inflamed populations. Two blood markers in particular were shown to change with acute exercise, tryptophan and TNF- α . Although tryptophan levels did decrease, it is difficult to make any sweeping conclusions, as the amount of total tryptophan does not account for the amount of free tryptophan in the blood [116]. Tryptophan at rest is largely bound to albumin, and therefore not able to be synthesized into serotonin within the brain. However, acute exercise has been shown to elevate free tryptophan in the brain through lipolysis via direct measurements in rat models [117], as well as indirect measurements in humans by exploring ratios of total tryptophan with other competing amino acids such as leucine and tyrosine [124]. Lipolysis rates increase with sustained exercise, where elevated levels of free fatty acids are released into the blood and displaces the binding of tryptophan to albumin, further elevating levels of free tryptophan [103, 117]. Therefore, even with the correlations with the whole sample between changes in tryptophan and negative mood sub-scores pre to post exercise, more analysis needs to be done to see if there is an increase in free tryptophan, and if this is correlated to these mood changes. Although the raw score for the ratio between kynurenine and tryptophan did not change with exercise, there was a trend for a correlation between a change in the ratio and changes in overall mood and depression, consequently signifying that a higher IDO expression leads to a higher depression score and a more negative mood. Due to the raw scores not changing, this may indicate that other mechanisms are in play.

As an alternate mechanism to changes in amino acids influencing acute mood changes after exercise there is the possibility that cytokines are impacting mood by acting on sites outside the kynurenine pathway. The only cytokine with both significant changes and at least a trending relationship to mood pre to post exercise was TNF- α . Higher levels of TNF- α have been shown in people with depression [10, 68, 125, 126] and when injected it has been shown to increase depressive symptoms [127]. This study showed that TNF- α decreased with exercise, indicating that there is an upstream action influencing this decline. TNF- α is locally released from monocytes, macrophages, and

adipose tissue and it has been shown to stimulate circulating IL-6, which in turn can stimulate various pro and anti-inflammatory responses [100, 120]. One of the responses is an increase in the anti-inflammatory cytokine IL-10, which in turn inhibits further production of TNF- α [128] and thus potentially explains the decline that was shown. Yet, while TNF- α was shown to decrease with exercise there was no change in IL-6 levels. It is possible that IL-6 levels were elevated within skeletal muscle, as more substantial elevations than that of serum have been shown in response to exercise [129]. If IL-6 was elevated in skeletal muscle it may explain the decline in TNF- α . However, the measurements of skeletal muscle IL-6 was beyond the scope of this study. An alternative inhibitor that may have been acting on TNF- α are the increases in cortisol and epinephrine. While this study did not measure these hormones, both have been shown to increase with exercise [100] and have an inhibiting effect on TNF- α [118, 130].

In regard to cytokines, the majority of correlations that were found in the subgroup between the pro-inflammatory cytokines and mood changes happened between pre to 1 hour post exercise. IL-6, IFN- γ , and TNF- α all had significant or trending correlations with tension or depression sub-scores during this time frame which suggests that cytokines are too slow to affect mood immediately, but instead play a role in maintaining a more positive mood afterwards. For these reasons, cytokines may still play a role in alterations of mood, but a much larger contributing physiological mechanism, such as cytokines acting on SERT proteins, remains evasive.

It may even be true that there are no physiological changes that are mediating mood, but instead it is purely psychological. Researchers argue that exercise may just serve as a distraction from other stressors in an individual's life [131, 132]. Another hypothesis is that exercise improves a person's mood because they feel in control of their life by doing it. Increasing one's self-efficacy may be enough to influence a positive mood change [112].

6.3.0 Limitations

Several limitations were apparent in this current study. First, the study was limited by lacking an able-bodied control group to compare with the chronically inflamed group. An able-bodied control group, could have assisted in establishing if the intensity of the exercise was sufficient to evoke a cytokine response or if the chronically inflamed populations had a blunted cytokine and amino acid response to the exercise session. Both the former and the latter would indicate that inflammatory blood markers may not play any role in acute mood changes if one of the groups had changes in inflammatory markers while both groups equally improved their moods from baseline.

Second, having subjects exercise at only a single intensity, means there is no way of knowing if the cytokine response is intensity-dependent and if that has any bearing on mood changes. Multiple studies have explored the variance in cytokines and intensity [122] and changes in mood and intensity in aerobic exercise [93, 111, 133], but none have combined the two. By undergoing aerobic exercise at multiple intensities, the establishment of how variations in changing moods may be mediated by cytokine alterations may be achieved.

Third, as discussed in the future directions section, the lack of anti-inflammatory cytokines in this thesis make it difficult to conclude if these cytokines play a role in acutely improving mood through moderate intensity exercise.

Fourth, this study attempted to measure the anti-inflammatory cytokine IL-10 as well as the pro-inflammatory cytokine IL-1 β but the data was not obtained due to technical difficulties.

Finally, the smaller sample size may have restricted the study's ability to obtain significant changes because of the high standard deviations amongst the group. While the sample size is normal for exercise in special populations, adding additional participants into a future study could strengthen the relationships that may exist between mood changes due to exercise and the inflammatory mechanisms behind them.

6.4.0 Future Directions

This study shows that moderate aerobic exercise is sufficient to evoke a positive change in mood in chronically inflamed populations. Correlations between mood sub-scores and cytokines, but not amino acids, indicate that cytokines may play a role in acute mood alterations due to exercise, but do not appear to act through the Kynurenine Pathway. Therefore, future research should explore other potential physiological mechanisms that may be facilitating these changes.

Other potential mechanisms that could play a role in these changes may include exercise induced changes in SERT transporters, endocannabinoids, anti-inflammatory cytokines, and the changing ratio of neuroprotective kynurenic acid to kynurenine.

First, SERT proteins increase reuptake of serotonin after release and have been shown to be upregulated by pro-inflammatory cytokines such as TNF- α and IFN- γ . This causes lower levels of extracellular serotonin in the brain. As SERT has been shown to be downregulated by anti-inflammatory cytokines such as IL-10 and IL-4 [77, 78], it is possible that decrements in levels of pro-inflammatory cytokines that were seen with TNF- α and the potential increase in anti-inflammatory cytokines due to acute exercise may decrease the rate of reuptake by SERT transporters and lead to an overall increase in serotonin within the brain and by extension, an improvement in mood. Future research should explore the relationship between acute exercise, SERT, and mood changes.

Second, future research should further explore the role of endocannabinoids and their influence on mood alterations. Traditionally it has been well-accepted that the increase in β -endorphin levels during exercise was the mechanism behind the common analgesic effects and elevated mood that has been shown in the literature. However, it has since been shown that this is not the case, as endorphins are unable to cross the BBB and therefore do not act centrally. [134]. An alternative explanation to this phenomenon are endocannabinoids, which use similar receptors. Anandamide, a fatty acid derivative, is the main endocannabinoid that binds to central CB1 and peripheral CB2 receptors, and can pass through the BBB (and therefore act centrally), as it is lipophilic. These receptors also have a high affinity for the psychoactive component of marijuana, THC. Endocannabinoid levels have been shown to increase after acute exercise [134–136] in an intensity-dependent manner [137]. Anandamide has been shown to be positively

correlated to changes in BDNF during acute exercise. As BDNF contributes to neurogenesis within the hippocampus in chronic exercise, and with the anti-depressant effect of acute exercise [136]. Further research is required to determine if exercise-induced changes in anandamide exist in those with SCI and MS, and if so, whether or not said changes account for the exercise-induced changes in mood.

Third, while this study did not show any significant changes in kynurenine levels with exercise, it is possible that while the levels of kynurenine remained unchanged, the ratio between the neuroprotective and neurotoxic alternative pathways may have shifted towards the neuroprotective pathway. The final product of the neuroprotective pathway is kynurenic acid. Aerobic exercise has been shown to stimulate PGC-1 α expression from skeletal muscle [103, 104]. PGC-1 α has also been shown to increase the expression of the enzyme kynurenine aminotransferase (KAT), thereby shifting the pathway towards kynurenic acid production. This shift would be considered neuroprotective since kynurenic acid is an NMDA receptor antagonist and thus a protector against stress induced depression. Further research should explore the ratio change of KYN: KYNA and its relationship with mood improvements after moderate exercise.

Fourth, this study did not measure changes in any anti-inflammatory cytokines. It is possible that instead of a correlation between improved mood and a decline in pro-inflammatory cytokines and amino acids, the role of increasing anti-inflammatory cytokines with exercise may be correlated the mood improvement that was shown in this study. Exercise has a unique anti-inflammatory profile distinctive from that of sepsis, in that there is not a rise in pro-inflammatory cytokines, but instead an increase in anti-inflammatory cytokines such as IL-6, IL-10, and IL-1ra [100, 120]. These cytokines work to inhibit the release of pro-inflammatory cytokines and may play a larger role in the improvement of mood and therefore should be examined in future analyses.

Fifth, future researchers should also explore the differences between able-bodied and chronically inflamed populations and how they each respond to moderate, aerobic exercise. Dysfunction within multiple systems that may be apparent in chronically inflamed populations could play a role in physiological differences that occur during exercise that explain why healthier populations may improve their mood more consistently than chronically inflamed populations.

In conclusion, there are many other potential physiological mechanisms that may contribute to the observed mood changes with exercise. They may all play some small role in contributing to these changes, but until these studies are completed and repeated, they will remain only hypotheses.

6.5.0 Conclusion

The current study was the first to examine the potential effects of acute exercise on mood and the role of cytokines as a mechanism accounting for the mood changes in people with multiple sclerosis and spinal cord injury. The results provided evidence that moderate exercise is sufficient in improving the mood in these populations. It appears that the Kynurenine Pathway does not play a role in exercise induced mood improvements, but a decline in pro-inflammatory cytokines may assist in the maintenance of a positive mood. Therefore, future research is warranted to explore the potential cytokine-related physiological mechanisms that may contribute to alterations in mood due to moderate intensity exercise.

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Appendices

Appendix A – Raw Data

1) POMS Scores

	TMD			Tension			Depression
Subject	PRE	POST	IHPOST	PRE	POST	IHPOST	PRE
1	44	24	21	11	3	1	11
2	49	34	48	14	8	10	8
3	72	44	49	9	6	9	10
4	3	9		0	3		0
5	57	30	18	10	1	1	14
6	-6	9	1	2	2	1	0
7	40	20	23	3	2	5	5
8	27	18	14	6	3	3	2
9	15	2	4	5	2	1	4
10	9	-3	-9	7	1	2	0
11	24	19	7	6	5	0	2
12	74	80	78	10	13	11	17
13	14	5	5	2	1	1	1
Average	32.46	22.38	21.58	6.54	3.85	3.75	5.69
SD	25.82	21.87	24.97	4.14	3.46	4.00	5.76

		Anger		Fatigue		Confusion	
POST	IHPOST	PRE	POST	IHPOST	PRE	POST	IHPOST
7	2	6	5	4	3	6	6
3	4	9	3	3	11	5	5
6	6	7	5	9	13	7	5
0		0	0		0	4	2
9	5	3	2	0	6	1	0
0	0	3	3	0	0	5	5
3	2	7	0	0	10	3	2
0	0	0	0	0	7	5	4
0	0	2	0	0	0	0	5
0	0	0	0	0	4	5	0
2	2	2	2	3	5	5	4
21	24	20	17	19	10	11	10
0	0	0	0	0	5	1	0
3.92	3.75	4.54	2.85	3.17	5.69	5.54	4.58
5.98	6.72	5.58	4.65	5.69	4.37	4.96	5.53

Vigour		
PRE	POST	IHPOST
17	23	17
18	24	15
1	7	7
19	20	
6	9	13
31	26	26
17	19	15
13	14	16
21	24	21
23	29	31
16	19	25
18	16	18
17	19	18
16.69	19.15	18.5
7.34	6.41	6.45

2) Blood Markers

Kynurenine (µmol/L)			Tryptophan (µmol/L)			
Subject	PRE	POST	IHPOST	PRE	POST	IHPOST
1	1.52	1.50	1.17	64.78	52.36	48.46
2	4.10		3.23	128.73	91.26	117.70
3	2.02	1.50	1.40	82.34	64.34	68.96
4	4.07	3.43		88.20	71.93	
5	1.51	3.11	3.4	94.05	67.80	87.01
6	1.9	1.39	2.00	77.42	73.44	108.80
7	2.63	2.06	2.94	112.23	84.59	116.80
8	2.88	1.63	3.23	111.43	118.00	114.03
9	3.70	2.70	3.75	148.87	127.80	105.15
10	2.91	4.72	3.57	112.37	96.80	153.05
11	2.03	2.93	1.95	81.09	65.52	80.69
12	3.88	10.17	2.99	93.34	109.49	93.37
13	6.17	6.88	2.63	104.25	115.75	97.35
Average	3.02	3.50	2.69	99.93	87.62	99.28
SD	1.33	2.64	0.86	22.92	24.20	26.83

IL-6 (pg/ml)			TNF- α (pg/ml)			INF- γ (pg/ml)		
PRE	POST	IHPOST	PRE	POST	IHPOST	PRE	POST	IHPOST
17.68	15.22	14.93	33.26	30.43	24.85	67.47	47.43	53.29
9.11	8.01	9	33.02	30.77	31.21	47.04	49.81	44.85
12.11	12.11	11.72	40.28	38.2	35.48	61.76	59.78	61.66
13.17	12.52		30.77	28.45		29.2	31.88	
8.34	8.01	7.1	26.91	26.38	22.42	34.25	33.49	32.7
11.09	10.78	11.72	29.35	30.77	26.31	47.04	43.13	46.03
12.67	12.35	13.85	25.92	25.02	23.97	24.95	24.65	27.45
6.92	4.92	10.78	23.22	22.11	21.14	42.65	43.61	41.64
			20.56	20.68	15.18			
6.26	7.26	8.41	23.22	23.78	18.66	36.36	32.7	34.25
8.53	8.34	8.14	14.42	11.71	12.46	34.98	32.9	31.24
8.34	8.01	7.5	10.61	10.9		34.43	28.71	26.38
7.34	6.56	5.82				28.22	23.41	26.65
10.13	9.51	9.91	25.97	24.93	23.17	40.7	37.63	38.74
3.31	3.03	2.91	8.3	7.91	6.94	13.19	11.07	11.73

Appendix B – EDSS Scores

	Kurtzke Expanded Disability Status Scale (EDSS)
0	Normal neurological exam (all grade 0 in Functional Systems (FS); cerebral grade 1 acceptable).
1	No disability, minimal signs in one FS (i.e., one grade 1 excluding cerebral grade 1).
1.5	No disability, minimal signs in more than one FS (more than one grade 1 excluding cerebral grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 0 or 1), or mild disability in three or four FS (three-four FS grade 2, others 0 or 1).
3.5	Fully ambulatory but with moderate disability in one FS (one grade 3 and one or two FS grade 2) or two FS grade 3, others 0 or 1, or five FS grade 2, others 0 or 1.
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters (0.3 miles).
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability. (Usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters (975 ft.).)
5.0	Ambulatory without aid or rest for about 200 meters (650 ft.); disability severe enough to impair full daily activities (e.g., to work full day without special provisions). (Usual FS equivalents are one grade 5 alone (others 0 or 1); or combinations of lesser grades usually exceeding specifications for step 4.0.)
5.5	Ambulatory without aid or rest for about 100 meters (325 ft); disability severe enough to impair full daily activities. (Usual FS equivalents are one grade 5 alone (others 0 or 1); or combinations of lesser grades usually exceeding specifications for step 4.0.)
6.0	Intermittent or constant unilateral assistance (cane, crutch, or brace) required to walk about 100 meters (325 ft.) with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)
6.5	Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters (65 ft.). (Usual FS equivalents are combinations with more than two FS grade 3+.)
7.0	Unable to walk beyond about 5 meters (16 ft.) even with aid, essentially restricted to wheelchair, wheels self in standard wheelchair a full day and transfers alone; up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone.)
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfers, wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+.)
8.0	Essentially restricted to bed or chair or perambulated in wheelchair; but may be out of bed much of the day; retains may self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
8.5	Essentially restricted to bed for much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally grade 4+ in several systems.)
9.0	Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4.)
9.5	Totally helpless bed patient; unable to communicate or effectively eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+.)
10	Death due to MS.

Abbreviation	Functional System
BB	Bowel & Bladder
BS	Brainstem
Cb	Cerebral or Mental
C11	Cerebellar
O	Other
P	Pyramidal (motor function)
S	Sensory
V	Visual

Functional Systems of the EDSS Scale

Appendix C – AIS Scale

STANDARD NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY

MOTOR

KEY MUSCLES

	R	L
C2		
C3		
C4		
C5		
C6		
C7		
C8		
T1		
T2		
T3		
T4		
T5		
T6		
T7		
T8		
T9		
T10		
T11		
T12		
L1		
L2		
L3		
L4		
L5		
S1		
S2		
S3		
S4-5		

Elbow flexors
Wrist extensors
Elbow extensors
Finger flexors (distal phalanx of middle finger)
Finger abductors (little finger)

0 = total paralysis
1 = palpable or visible contraction
2 = active movement, gravity eliminated
3 = active movement, against gravity
4 = active movement, against some resistance
5 = active movement, against full resistance
NT = not testable

Hip flexors
Knee extensors
Ankle dorsiflexors
Long toe extensors
Ankle plantar flexors

☐ Voluntary anal contraction (Yes/No)

TOTALS ☐ + ☐ = ☐ **MOTOR SCORE**
(MAXIMUM) (50) (50) (100)

SENSORY

KEY SENSORY POINTS

	R	L
C2		
C3		
C4		
C5		
C6		
C7		
C8		
T1		
T2		
T3		
T4		
T5		
T6		
T7		
T8		
T9		
T10		
T11		
T12		
L1		
L2		
L3		
L4		
L5		
S1		
S2		
S3		
S4-5		

0 = absent
1 = impaired
2 = normal
NT = not testable

Any anal sensation (Yes/No) ☐

TOTALS ☐ + ☐ = ☐ **PIN PRICK SCORE** (max: 112)
(MAXIMUM) (56) (56) (56) (56)

☐ + ☐ = ☐ **LIGHT TOUCH SCORE** (max: 112)

NEUROLOGICAL LEVELS

The most caudal segment with normal function

R L

SENSORY ☐ ☐

MOTOR ☐ ☐

COMPLETE OR INCOMPLETE? ☐

Incomplete = Any sensory or motor function in S4-5

ASIA IMPAIRMENT SCALE ☐

ZONE OF PARTIAL PRESERVATION

Partially innervated segments

R L

SENSORY ☐ ☐

MOTOR ☐ ☐

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Version 4j
CHC 1994

Appendix D – Profile of Mood States

Profile of
Mood States
- POMS

*Directions: Describe HOW YOU FEEL RIGHT NOW
by circling the most appropriate number after each of the words listed below:*

FEELING	Quite a				
	Not at all	A little	Moderate	bit	Extremely
1. Friendly	1	2	3	4	5
2. Tense	1	2	3	4	5
3. Angry	1	2	3	4	5
4. Worn Out	1	2	3	4	5
5. Unhappy	1	2	3	4	5
6. Clear-headed	1	2	3	4	5
7. Lively	1	2	3	4	5
8. Confused	1	2	3	4	5
9. Sorry for things done	1	2	3	4	5
10. Shaky	1	2	3	4	5
11. Listless	1	2	3	4	5
12. Peeved	1	2	3	4	5
13. Considerate	1	2	3	4	5
14. Sad	1	2	3	4	5
15. Active	1	2	3	4	5
16. On edge	1	2	3	4	5
17. Grouchy	1	2	3	4	5
18. Blue	1	2	3	4	5
19. Energetic	1	2	3	4	5
20. Panicky	1	2	3	4	5
21. Hopeless	1	2	3	4	5
22. Relaxed	1	2	3	4	5
23. Unworthy	1	2	3	4	5
24. Spiteful	1	2	3	4	5
25. Sympathetic	1	2	3	4	5
26. Uneasy	1	2	3	4	5
27. Restless	1	2	3	4	5
28. Unable to concentrate	1	2	3	4	5
29. Fatigued	1	2	3	4	5

30. Helpful	1	2	3	4	5
31. Annoyed	1	2	3	4	5
32. Discouraged	1	2	3	4	5
33. Resentful	1	2	3	4	5
34. Nervous	1	2	3	4	5
35. Lonely	1	2	3	4	5
36. Miserable	1	2	3	4	5
37. Muddled	1	2	3	4	5
38. Cheerful	1	2	3	4	5
39. Bitter	1	2	3	4	5
40. Exhausted	1	2	3	4	5
41. Anxious	1	2	3	4	5
42. Ready to fight	1	2	3	4	5
43. Good-natured	1	2	3	4	5
44. Gloomy	1	2	3	4	5
45. Desperate	1	2	3	4	5
46. Sluggish	1	2	3	4	5
47. Rebellious	1	2	3	4	5
48. Helpless	1	2	3	4	5
49. Weary	1	2	3	4	5
50. Bewildered	1	2	3	4	5
51. Alert	1	2	3	4	5
52. Deceived	1	2	3	4	5
53. Furious	1	2	3	4	5
54. Effacious	1	2	3	4	5
55. Trusting	1	2	3	4	5
56. Full of pep	1	2	3	4	5
57. Bad-tempered	1	2	3	4	5
58. Worthless	1	2	3	4	5
59. Forgetful	1	2	3	4	5
60. Carefree	1	2	3	4	5
61. Terrified	1	2	3	4	5
62. Guilty	1	2	3	4	5
63. Vigorous	1	2	3	4	5
64. Uncertain about things	1	2	3	4	5
65. Bushed	1	2	3	4	5

Appendix E – Ethical Approval



Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 10/28/2016
PRINCIPAL INVESTIGATOR: DITOR, David - Kinesiology
FILE: 16-073 - DITOR
TYPE: Masters Thesis/Project STUDENT: Scott Donia
SUPERVISOR: David Ditor
TITLE: The effects of acute aerobic exercise on mood in individuals with multiple sclerosis and incomplete spinal cord injury

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW

Expiry Date: 10/31/2017

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **10/28/2016** to **10/31/2017**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 10/31/2017. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at <http://www.brocku.ca/research/policies-and-forms/research-forms>.

In addition, throughout your research, you must report promptly to the REB:

- a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
- c) New information that may adversely affect the safety of the participants or the conduct of the study;
- d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Appendix F – Pilot Data

A pilot study preceded the events of this thesis to determine the acute effects of aerobic exercise on mood and pain in individuals with multiple sclerosis (MS) and spinal cord injury (SCI). 11 participants (6 MS and 5 SCI) completed 30 minutes of moderate aerobic exercise. Participants maintained a cadence of 100 strides per minute, while altering the resistance level to sustain a 13 on a 6-20 rate of perceived exertion scale. Prior to exercise, participants completed 4 questionnaires and completed them again post exercise. Two of the questionnaires (Feeling Scale and Profile of Mood States) pertained to changes in mood, while the other two (Brief Pain Inventory and Neuropathic Pain Questionnaire) concerned changes in pain.

Following the bout of exercise, there was a significant improvement in mood as shown by a reduction in the total POMS questionnaire score from pre to post-exercise (28.64 ± 18.64 to 15.82 ± 14.54 , $p=.01$). Subscale analysis showed significant reductions in the Tension (4.55 ± 3.14 to 2.82 ± 0.75 , $p=.02$), Depression (5.18 ± 5.56 to 1.36 ± 2.25 , $p=.01$), Anger (4.18 ± 4.69 to 1.45 ± 2.46 , $p=.01$), and Confusion (6.36 ± 3.17 to 4.18 ± 3.89 , $p=.04$) components of the POMS from pre to post exercise. There was no change in Feeling Scale (3 ± 1.34 to 3.36 ± 1.03 , $p=.48$) Brief Pain Inventory (3 ± 2.22 to 2.93 ± 2.03 , $p=.94$; 2.71 ± 2.22 to 2.51 ± 2.17 , $p=.83$; 2.45 ± 2.58 to 1.91 ± 2.12 , $p=.22$) or Neuropathic Pain (14.09 ± 13.98 to 10.87 ± 11.59 , $p=.56$; 25.85 ± 26.05 to 25.34 ± 25.56 , $p=.96$; 16.82 ± 23.29 to 13.97 ± 18.42 , $p=.75$). This study showed that moderate intensity exercise was sufficient in improving mood states but not changes in pain and thus viable to explore potential mechanisms behind these exercise-induced alterations in mood.

Table #10: Pilot Participant Characteristics

MS/SCI	TYPE	EDSS/ AISA	SEX	AGE (YEARS)	YEARS POST (YEARS)	LEVEL ACHEIVED
MS	SPMS	6.0	F	59	31	3
MS	SPMS	6.0	F	57	25	4
MS	RRMS	5.0	F	45	3	4
MS	RRMS	4.0	F	41	8	1
MS	SPMS	6.0	M	55	22	1
MS	SPMS	6.0	F	59	41	2
SCI	C2	D	M	65	1	5
SCI	C5	D	M	69	7	2
SCI	T10	A	M	60	5	5
SCI	L4	D	M	60	7	4
SCI	C3	D	M	59	2	4
AVE				57.2	13.8	3.2
SD				8.0	13.6	1.5

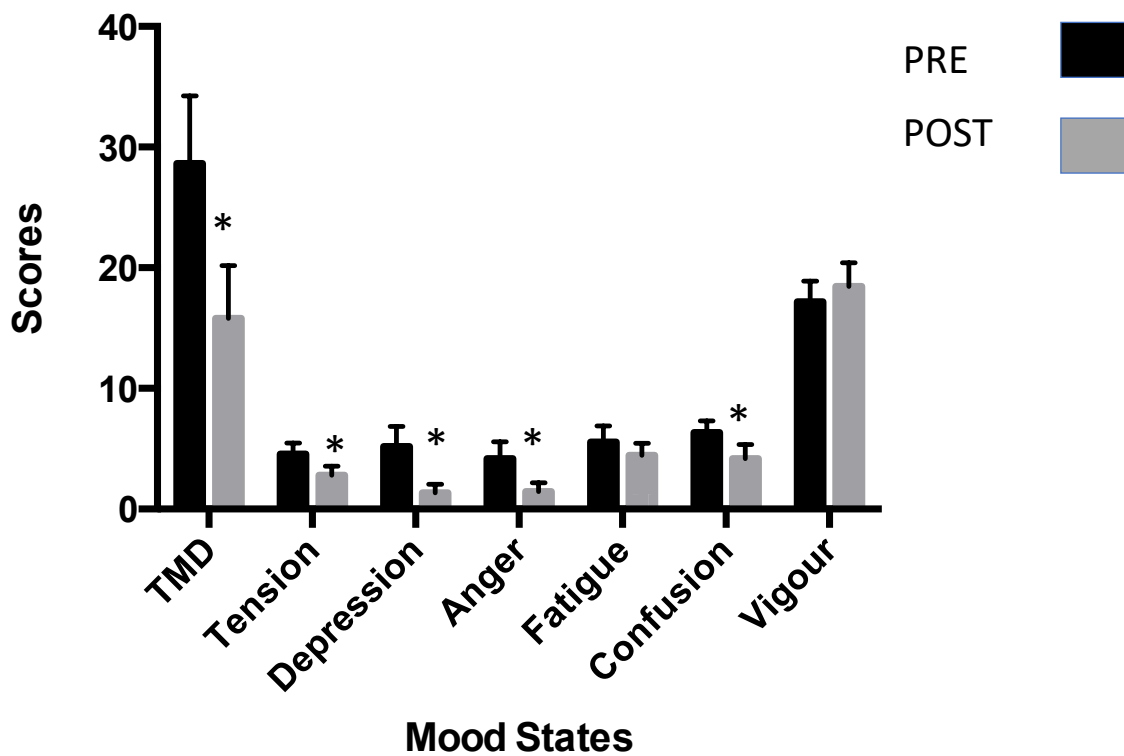


Figure #7: Pilot Profile of Mood States

Figure #7: Pilot Profile of Mood States

Table #11: Pilot Feeling Scale Questionnaire

	Pre	Post	Paired T-Test
Feeling Scale	3.00±1.34	3.36±1.02	.48

*All results are shown as mean ± SD. *Significantly different with $P < 0.05$*

Table #12: Pilot Brief Pain Inventory

	Pre	Post	Paired T-Test
Severity	3.00±2.22	2.93±2.03	.94
Intensity	2.71±2.22	2.51±2.17	.83
Right Now	2.45±2.58	1.91±2.12	.22

*All results are shown as mean ± SD. *Significantly different with $P < 0.05$*

Table #13: Pilot Neuropathic Pain Questionnaire

	Pre	Post	Paired T-Test
Sensory	14.08±13.98	10.87±11.59	.56
Affect	25.85±26.05	25.34±25.56	.96
Sensitivity	16.82±23.29	13.97±18.42	.75

*All results are shown as mean ± SD. *Significantly different with $P < 0.05$*